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ERRATA

Page 90, first line of citation no. 26, for "or" read of.
Page 154, third line of citation no. 24, for "nedelandsch" read Nederlandsch.
Page 184, line 16, for "confined" read confirmed.
Page 200, first column of table III, fourth line, for "A. M. 21" read A. M. 22.
Page 427, line 8, for "decreases" read decrease.

Bind in place

JULIUS VON SACHS
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To

JULIUS VON SACHS

BORN OCTOBER 2, 1832

THIS VOLUME OF PLANT PHYSIOLOGY IS DEDICATED

IN HONOR OF

THE ONE-HUNDREDTH ANNIVERSARY OF HIS BIRTH

PLANT PHYSIOLOGY

JANUARY, 1932

WILTING AND WITHERING OF GRASSES IN GREENHOUSE CULTURES AS RELATED TO WATER-SUPPLYING POWER OF THE SOIL[1]

J. Dean Wilson and Burton E. Livingston

(WITH FOUR FIGURES)

Introduction

It has recently been emphasized (**14, 15, 19, 20, 32**) that the two main environmental conditions that influence the development and health of ordinary leafy plants, as far as water relations are concerned, are (a) the capacity of the soil to deliver water to the plant roots and (b) the capacity of the aerial surroundings to permit water loss from the foliage through transpiration. The second of these conditions may be measured and recorded in a fairly satisfactory way, by means of atmometers of various forms, and numerous studies on the relations between evaporation and plant health have been reported in the literature of plant physiology and ecology. While very much more remains to be learned about the influence of this complex climatic feature on plant activity, it is safe to say that our knowledge of evaporation relations and our methods for securing instrumental records of fluctuations in the intensity of evaporation conditions are now far more advanced than are our knowledge and methods with regard to the influences exerted on plants by soil-moisture conditions. What happens to an ordinary plant in the region below the soil surface still remains a subject for rather free conjecture. Soil science furnishes much that is relatively definite, consistent and apparently reliable in its field, but the points of contact between our knowledge of plant physiology and our knowledge of soil physics and soil chemistry are still comparatively few and are generally unsatisfactory. The soil system is so complex that it has not readily lent itself to the usual analytical methods and our present great fund of information about soil moisture leaves us still unable to integrate the many separate portions of that information in any way that would permit clear insight into the water relations of root systems. It appears that the analytical methods that have yielded valuable principles and generalizations in the

[1] Botanical contribution from the Johns Hopkins University, no. 92.

fields of physics and chemistry are not as yet very promising when we aim to study the relations between two such very complex systems as the plant and the soil. To further such a study it is essential that primary attention be given to the soil system considered as a whole, avoiding the distractions of detail that confound us along the paths of the more thoroughly analytical method as generally employed.

Roots are, of course, unable to absorb any water that has not already somehow come into contact with their absorbing surfaces, and the maximum rate at which a given root system may absorb water can not surpass the rate at which root surfaces and soil moisture come into contact. If for the moment we consider the absorbing surfaces of the root system as fixed in position and extent, then the maximum rate of absorption is limited at any instant by the rate at which the soil system can supply water to the root-soil surfaces. Conditions within the plant should control the rate of absorption as long as the latter is slower than the highest possible rate of external supply, but soil conditions should control the absorption rate whenever the soil system is unable to bring water to the absorbing surfaces as rapidly as it would be absorbed if the rate of supply were more rapid. Consequently the primary soil condition that may limit the rate of entry of water into stationary roots is the rate at which the soil system can deliver water to these organs. This has been called the water-supplying power of the soil (**2, 5, 6, 11, 12, 16, 17, 18,** [p. 115], **22, 32**), that is, its capacity as a mechanism, to deliver water at a unit of water-absorbing surface.

This dynamic characteristic of the soil around the roots of a plant is, of course, the resultant of secondary, contributory or component soil characteristics, such as the kind of soil (measured as its water-holding power, its moisture equivalent, etc.) and its state of packing, its water content per unit of natural volume, and its temperature. These are static characteristics that may be more or less satisfactorily measured from time to time, but measurements of them are of very slight value in physiology, ecology, agronomy, horticulture, sylviculture, etc., because it is as yet impossible to deduce from such measurements a numerical index of water-supplying power for any time period. Such an index should differ in magnitude with the kind of soil, with its degree of packing, with its moisture content, with its temperature, and perhaps with other static features. Its indirect derivation, for a given soil region, from the results of measurements of these components taken separately must consequently remain complex and tedious in the extreme.

Direct measurement of the water-supplying power of the soil near the roots of the plant appears to offer a promising means by which we may hope to improve an understanding of the dynamic water relations between

plant and soil. The only feasible and tested method thus far available for securing direct measurements of this dynamic soil feature is the soil-point method first brought forward by LIVINGSTON and KOKETSU (**17**). A suitable artificial absorbing surface, of porous porcelain (or wood), is applied to the soil at the depth considered and the capacity of the soil to supply water is measured as the amount of water actually absorbed in a unit of time. The water-supplying power is therefore the rate at which the given soil, at the given depth, is capable of delivering water to a stationary absorbing surface. This is a dynamic variable, the magnitude of which fluctuates. It does not directly influence the actual rate of water absorption by a root unless the absorbing capacity of the root surface exceeds it in magnitude, when it acts to limit the rate of absorption. Direct soil-point measurements of the water-supplying power of the soil may be made at suitable intervals, at any required depths in the soil, and graphs may be constructed from them to show fluctuations in water-supplying power throughout a period of time. The operations are relatively very simple and rapid (though the method is surely susceptible of further improvement) and no intricate calculations are required. The soil mechanism itself is allowed to integrate the several component terms or factors or arguments (in the sense of the mathematical calculus) and the data secured are measurements of the resultant only. The observer measures directly the function of several variables instead of measuring the variables and then attempting to calculate the value of the function from the results of his measurements.

The soil points used in this study were like those employed by WILSON (**32**). The instrument is a hollow porous-porcelain piece with wall about 3 mm. thick. It has a cylindrical part open at the top and continuous below with a conical part that ends in a sharp point. The cylindrical part is 2.4 cm. in outside diameter and 2.5 cm. high. The outer surface is thoroughly waterproofed near the point, also throughout the cylindrical part and the upper portion of the conical part, and a band 2 cm. high on the conical part is left uncoated. This zone has a diameter of about 2.2 cm. at its upper margin. It presents to the soil into which the instrument is thrust an absorbing surface of about 12 sq. cm.

It has been mentioned or implied by PULLING and LIVINGSTON (**25**), PULLING (**24**), WILSON (**32**), and VEIHMEYER (**27, 28**) that the initial water-supplying power of the soil adjacent to a water-absorbing surface (such as that of plant root or artificial absorber) is not maintained in the case of aerated soils with water content below maximum field capacity unless nearly saturated soil is closely adjacent to the soil region considered. When an absorbing surface with sufficient capacity for water absorption is brought into capillary contact with moist soil the rate of water delivery from the soil is initially at a maximum and rapidly decreases. The water nearest to

the absorber is taken up promptly but the rate of subsequent absorption is limited by the rate at which water from more distant soil films migrates to the absorber. This rate of migration becomes very slow, as has been emphasized by VEIHMEYER, when the additional water must move a considerable distance before being absorbed, and the water-supplying power of the soil therefore decreases rapidly as absorption proceeds from such soils. It may happen in some cases that water distillation from soil films to root surface is of some importance in maintaining health with low transpiration rates, but it seems improbable that plant health would generally be long maintained without movement of liquid water from soil to root.

There seems to be no reason to doubt that roots in moist soils experience this progressive decrease in the dynamic availability of water adjacent to their absorbing surfaces. When an absorbing surface of a root initially comes into contact with a new system of soil films, by the penetration of root tip or root-hair into untouched soil, absorption may be relatively rapid and the water-supplying power of the soil system may be greater for a time than the absorbing power of the root surface. If absorption continues, however, supposing the absorbing power to remain the same as at first, there eventually comes a time when the supplying power of the soil is smaller than the absorbing power of the root surface, and the supplying power of the soil then becomes the limiting condition for the process of water absorption. It follows, as has been recently emphasized by VEIHMEYER, WILSON and others, that a root system in a soil that is merely moist and does not become wetter can generally neither maintain nor increase an already considerable rate of water absorption unless the roots continue to advance into new soil at an adequate rate.

It is probable that one of the effects of the onset of inadequacy in the water-supplying power of the soil about the roots of a plant is generally a retardation leading toward the ultimate cessation of root advance, for root growth and the formation of root-hairs requires additional water, and water that has been fixed by growth may be considered as temporarily held in the turgid tissues, being therefore generally removed from consideration. If water can move at an adequate rate into the growing region of root or root-hair, from the older regions (perhaps ultimately from some water-storage tissue of the plant or from the absorbing surfaces of another portion of the root system that may still absorb water more rapidly than it is conducted to other parts), then it should be possible for some root and root-hair growth to continue after the water-supplying power of the adjacent soil has become too low to support root growth.[2] It may even sometimes happen that a root

[2] HENDRICKSON and VEIHMEYER (8) report that this sort of root growth was not observed in experiments planned to throw light on some of the possibilities here mentioned.

may elongate at the expense of water of respiration (**1**), the root surfaces continuing to advance slowly into new soil regions where the water-supplying power is already inadequate. (Of course water of respiration does not enter the root at all; it is chemically formed in the tissues through the oxidation of organic material that has migrated hither from some other part of the plant.) But it appears highly probable that inadequacy in the capillary water-supplying power of the soil adjacent to the absorbing portions of a root system generally soon makes itself effective on the plant by retarding or stopping root enlargement (**8**). If this represents a general truth, then the continuous growth of roots is of basic importance for the maintenance of the rate of water absorption from soils that are characterized by relatively low water-supplying power, a consideration that does not thus far appear to have been seriously considered in the discussion of soil moisture conditions and water absorption by plants.

WEAVER (**30**, p. 44), in discussing root growth in field crops, mentions that root-hair growth makes fresh stores of water and solutes available to the plant and calls attention to the fact that but few root-hairs are produced by field crops in wet soils, while, if the plants do not wilt meanwhile, the greatest development of root-hairs may occur with soil-moisture contents somewhat less than would give the highest crop yields. It may be that some plants with slightly inadequate water supply (in their apparent "endeavor" to reach new sources of water) may produce root-hairs and small roots at the expense of the rest of the plant, with respect to water as well as with respect to other materials. This may act to hinder crop production and may thus limit the final yield to some extent.

The importance of the actual process of root enlargement is probably very great for upland plants, especially in times of more or less pronounced drouth. At such times the water-supplying power of the soil is relatively low and the wiping-up action of advancing roots and root-hairs should be very essential to the continued development of the plant. Such a plant can not long maintain its vigor and at the same time wait for the soil system to deliver water to its absorbing surfaces; these surfaces must be extended rapidly into less depleted regions of the soil, or else growth must soon become greatly retarded or cease entirely. It consequently follows that the instrumentally determined indices of water-supplying power that are requisite for the dynamic study of plant water relations should represent the *initial* water-supplying power for each test; that is, they should represent the condition encountered by a growing root or root-hair when it *first* comes into the soil region considered.

The soil-point method is especially suited to the determination of these initial values. As recently employed, this method gives values of the

supplying-power index that represent only the first hour after an absorbing surface is brought into capillary contact with the soil surface tested. Consecutive tests may, of course, be made by applying several instruments, one after another to the same soil surface, and valuable information may thus be secured regarding the rate of decrease in the water-supplying power as absorption proceeds.

If a soil is very wet, water absorption may continue for a long time after the absorbing surface of a growing root comes into contact with any particular portion of soil. With drier soils this period of absorption is limited, as has been said, and if the soil is sufficiently dry no absorption may occur. The period during which absorption may occur is shorter the drier is the soil into which the absorbing surfaces are thrust, and some specific degree of dryness should correspond to the single-hour period employed in connection with the soil-point method. If a soil region is so dry that newly formed roots find its water-supplying power inadequate to their needs within an hour after they have made contact with the delivering surface, it is safe to say that such a soil region generally has a water-supplying power too low for successful development of the plant. Periods much shorter than one hour are inconvenient in the application of the soil-point method and periods much longer introduce more and more the danger that the porous porcelain of the instrument may become so wet within the period that its own absorbing power becomes the thing measured, rather than the water-supplying power of the soil. This undoubtedly occurs with very wet soils, but it probably does not generally occur with supplying powers of less than 1000 mg. per soil point for the single hour. As WILSON (**32**), MARSHALL (**21**) and others have shown, the one-hour period is well suited to the study of soil water-supplying powers in relation to plant health.

Before bringing this introduction to a close it is essential to mention again a point emphasized by WILSON, that the insertion of a soil point into the soil inevitably involves some alteration in the water-supplying power at the very surface where the test is to be made. The soil is compressed laterally and to some extent downward as the conical opening is made for the insertion of the instrument. Therefore, the soil adjacent to the instrument is generally somewhat different in water-supplying power from the unmodified soil. With progressively lower values of the supplying-power the effect of this artificial compression becomes less, however, and the artifact thus introduced may safely be considered as very small when the index values dealt with approach the critical magnitudes about which growth ceases, wilting begins, etc. In any event, the effect of artificial compression is always relatively small and appears to be quite negligible in comparison with the differences in water-supplying power observed for different places

at the same depth in the same soil mass. When greater precision becomes desirable in the measurement of water-supplying power the method may perhaps be modified so as to avoid or at least greatly diminish this error.

The present paper reports the results of a study of the progress of the wilting, withering and death of originally healthy and vigorous greenhouse plants in winter, after watering had been discontinued. The study dealt with the progressive decrease in the water-supplying power of the soil about the roots as the soil dried out. The experimentation was carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, in the fall and winter of 1925–26.

Most of the cultures used were in shallow trays or flats but a few tests were made with pot cultures. The three greenhouse rooms of the Laboratory of Plant Physiology were used, several cultures being placed in each room. The soils of the different flats and pots were all approximately alike. The climatic conditions were about the same for all the cultures in each greenhouse room and they were not very greatly different for the different rooms. The cultures in flats will be considered first in the following pages and a brief section will be devoted to the results secured with pot cultures.

Cultures in flats

Methods

Seventeen different grasses were planted in flats, two or more species in each flat. Two clovers commonly found in lawns were also added to the group. Each kind of plant occupied an area about 80 x 70 cm., and a similar area without plants served as a control for each greenhouse room. Each flat was filled with soil to a depth of about 18 cm., little attention being given to packing since these cultures were to be watered in the ordinary way for a period of several months before critical observations were to be begun. The soil used was a friable mixture, such as that commonly used in these greenhouses, made up of one part each of sand and leaf-mold and three parts of a rather heavy loam (Sassafras loam). The water-holding capacity of the mixture was, by the Hilgard method, 44 per cent. on the basis of dry volume, or 50 per cent. on the basis of dry weight. Enough soil was prepared to care for these cultures and also for the pot cultures that were to be started subsequently.

The flats were planted early in June, 1925, either by sowing seeds or planting stolons, and the plants were allowed to grow, with watering as needed, until December 30, when they were uniformly in excellent condition. The flats were all thoroughly watered by sprinkling on the last-named date and the two spheres of a radio-atmometer (**13**), one white and one lampblack-coated, were installed at corners of one of the flats in each

greenhouse room, each sphere being about 25 cm. above the soil surface. In the second (middle) room a Richard thermograph was placed in a shaded but well ventilated position, to record temperature fluctuations, which were practically the same in all three rooms.

On December 31, soil-point readings on these cultures were begun, for the 6-cm. depth, and these were continued at intervals of three days until February 14. The soil points used in these tests, which had been carefully selected for uniformity in weight and absorptive capacity, were dried in the air for two days after each determination and then placed in a desiccator until needed for use again. Two points were used for each observation and the reading was obtained by dividing by 2 the total gain in weight for the usual one-hour period of exposure in the soil. To prevent increased evaporation from the soil and other possible disturbances, the little holes left on removing the soil points were always immediately filled with soil from the reserve supply. At each time of observation a test was made for the soil areas without plants. The different plant areas in any flat were not considered separately until some of the plants began to show slight effects of drouth, on January 18. Throughout this preliminary period, while all the plants were still vigorous, a soil-point reading was secured for each of the ten flats at each time of observation. On January 18 and at later times of observation readings for all plant areas, as well as for the control area, were made at about the same time. These observations were continued at 3-day intervals, as has been said, throughout the period while the plants passed through various successive stages of drouth injury and until the leaves of all but two of the forms were dead, the two exceptions (red fescue and sheep's fescue) being very drouth resistant.

On February 14 the cultures were all again thoroughly watered and then kept well supplied with water throughout the following month, to ascertain whether the plants in each case would recover when an adequate water supply was again maintained. This treatment resulted in recovery such that red fescue, sheep's fescue, Kentucky blue-grass and Canadian blue-grass were in good condition on March 14. With awnless brome-grass, tall oat-grass and orchard grass there was no recovery, while all of the other forms tested had returned to fair condition by the end of this second period of watering.

The atmometers were read every three days, at each time when soil-point readings were taken, and the average daily, corrected water losses for the 3-day period, from the white and from the blackened sphere, were recorded for each room of the greenhouse. Corresponding averages for the mean temperatures for each 3-day period were secured from the thermograph tracings for the middle room. Also, the growth conditions of the corresponding plants were recorded when soil-point readings were made. Nu-

merical values or scores were given, referring to five different degrees of health, based on the apparent condition of the leaves, as follows: 4, plants in excellent condition; 3, condition good but not excellent; 2, condition poor, with leaves wilting; 1, badly wilted but partly recovered after 18 hours in a moist chamber; 0, badly wilted, with no recovery after 18 hours in a moist chamber, many of the leaves being gray and crisp. It was noted that these grass leaves, which wilted and withered in the greenhouse in the winter season, generally failed to develop the brown colors that are so characteristic of most of these forms when their leaves die from the effects of drouth in the open at Baltimore. The dead leaves of these greenhouse-grown grasses became greenish or silvery gray upon drying-up and remained so for some time afterwards. They became brown, however, after several weeks of subsequent exposure in the greenhouse. This difference may be due to difference in light effects, the summer light acting to alter the color of the leaves more rapidly.

An attempt was made to detect the beginning of a rather definite stage of bad wilting, similar to the stage called the beginning of "permanent wilting" by BRIGGS and SHANTZ (**3**). It will be remembered that permanent wilting is defined as a degree of wilting from which the plant fails to recover when its transpiration is practically stopped, but from which it recovers (although slowly in many cases) if given adequate water supply about its roots. BRIGGS and SHANTZ exposed their supposedly permanently wilted plants in a saturated atmosphere for an undefined period and recorded them as permanently wilted only when they failed to show recovery. CALDWELL (**4**) and SHIVE and LIVINGSTON (**26**) employed a 24-hour period for this moist-chamber test. In the present studies the test was applied for 18 hours (including the night period) instead of 24, by inverting over a group of the plants in question a glass crystallizing dish about 15 cm. in diameter and about 8 cm. in depth. The edge of the inverted dish was pressed firmly against the soil and the enclosed air-chamber must very soon have become practically saturated with water vapor. These tests thus consisted in surrounding the leaves with nearly water-saturated air for a period of approximately 18 hours, during which time there was no significant transpiration.

In most cases where the moist-chamber test just described was applied no recovery was noted at the end of the test period and it was considered that the plants that failed to recover had at least passed beyond the particular stage of wilting here used as approximately corresponding to the beginning of permanent wilting as defined by BRIGGS and SHANTZ. These plants were given the score value of 0, being classed as vegetatively dead. In some other cases considerable recovery (of about half or more of the leaves) was manifest at the end of the 18-hour period under the crystalliz-

ing dish and the plants showing such recovery were given the numerical score value of 1, very poor.

This method of determining the soil conditions coincident with the attainment of a particular stage of wilting might easily be made considerably more precise, especially if the soil-point readings were more frequently taken. However, as the plants approached the critical stage of wilting here considered, the rate of decrease in the water-supplying power of the soil was very slow. It is consequently probably safe to say that the plants given the growth-index value of 0 had not passed the critical wilting point by more than three days at most, and that no plants given the index value of 1 had yet reached that stage of wilting.

Plants used

Two species of clover and seventeen different grasses, the latter including nine different genera, were used. Some of these are common on lawns of the Baltimore region and elsewhere, while others are often used in agricultural practice. They are known to differ widely in general drouth resistance on lawns and in meadows and they consequently offer opportunity for making selections to suit different climates and soils. These nineteen forms were as follows: (**9, 23**) : Alsike clover (*Trifolium hybridum* L.), white clover (*Trifolium repens* L.), Kentucky blue grass (*Poa pratensis* L.), Canada blue grass (*Poa compressa* L.), red fescue (*Festuca rubra* L.), sheep's fescue (*Festuca ovina* L.), Chewing's fescue (*Festuca* sp.), Rhode Island bent grass (*Agrostis tenuis* Sibth.), velvet bent grass (*Agrostis canina* L.), Vermont bent grass (*Agrostis* sp.), Washington bent grass (*Agrostis* sp.), perennial rye-grass (*Lolium perenne* L.), Bermuda grass (*Capriola dactylon* Adans.), tall oat-grass (*Arrhenatherum elatius* L.) brome grass (*Bromus inermis* Leyss.), orchard grass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), redtop (*Agrostis palustris* Huds.), meadow fescue (*Festuca elatior* L.).

Numerical data

Our temperature and evaporation data are presented in table I and the corresponding soil-moisture and plant data are given in table II. The dates of observations are indicated across the top of the table in each case. The temperature values are given in degrees Centigrade, each value representing the average of the three daily means for the 3-day period indicated. The mean for each day was the average of the maximum and minimum for the day, as these were shown on the thermograph tracing, and the three daily means for each period were simply averaged to represent the 3-day period. Each evaporation value represents the average daily, corrected water loss (in cubic centimeters) from the blackened atmometer sphere (*E*) or from

TABLE I

MEAN DAILY TEMPERATURES AND CORRECTED EVAPORATION VALUES FOR GREENHOUSE CULTURES, FOR 3-DAY PERIODS, FROM DEC. 31, 1925, TO FEB. 14, 1926

3-DAY PERIOD ENDING	JAN. 3	JAN. 6	JAN. 9	JAN. 12	JAN. 15	JAN. 18	JAN. 21	JAN. 24	JAN. 27	JAN. 30	FEB. 2	FEB. 5	FEB. 8	FEB. 11	FEB. 14
Temperature (Deg. C.)															
Room II of greenhouse	18	19	15	16	15	16	21	15	16	16	18	17	17	17	19
Evaporation (cc.)															
Room I of greenhouse:															
Blackened sphere (*E*)	22.7	12.7	17.7	18.7	20.0	16.3	22.7	20.0	23.3	21.3	15.0	20.7	20.3	18.0	20.0
White sphere (*e*)	18.3	11.3	14.7	15.7	18.0	15.3	19.3	17.3	20.0	18.0	13.3	17.7	17.7	15.3	16.7
Sunshine effect (*E*–*e*)	4.3	1.3	3.0	3.0	2.0	1.0	3.3	2.7	3.3	3.3	1.7	3.0	2.7	2.7	3.3
Room II of greenhouse:															
Blackened sphere (*E*)	19.0	11.7	15.3	16.3	18.3	15.3	19.7	16.3	19.7	17.7	12.7	17.7	19.0	14.0	17.0
White sphere (*e*)	17.3	10.7	13.7	14.7	16.7	14.0	17.7	14.7	17.3	16.0	12.0	16.3	17.7	13.0	15.7
Sunshine effect (*E*–*e*)	1.7	1.0	1.7	1.7	1.7	1.3	2.0	1.7	2.3	1.7	0.7	1.3	1.3	1.0	1.3
Room III of greenhouse:															
Blackened sphere (*E*)	21.0	11.7	17.0	18.0	20.3	16.7	21.3	19.7	22.7	19.7	14.3	18.7	20.0	16.7	20.0
White sphere (*e*)	18.0	10.0	14.3	15.0	17.3	15.3	19.0	16.3	19.0	17.3	13.0	16.3	17.3	15.0	17.3
Sunshine effect (*E*–*e*)	3.0	1.7	2.7	3.0	3.0	1.3	2.3	3.3	3.7	2.3	1.3	2.3	2.7	1.7	2.7

the white sphere (e), for the 3-day period. It was secured by dividing by 3 the corrected total loss for the 3 days in each case. Each actual loss observed was, of course, first multiplied by the coefficient of the atmometer sphere from which the loss had occurred. The value for the sunshine effect ($E-e$) for each 3-day period is shown (in cubic centimeters) as the difference between the mean daily corrected loss from the blackened sphere and the corresponding mean daily loss from the white sphere. These atmometer data are arranged in three groups, for the first, second and third rooms of the greenhouse.

The data for the initial water-supplying power of the soil (table II) are expressed in terms of milligrams of water absorbed per soil point, for the 1-hour period and for the 6-cm. depth. Each index value represents the number of milligrams supplied to the soil point, across a surface of about 12 sq. cm. Thus an index value of 100 indicates that the soil could supply water to the absorbing surface at a rate of about 8 mg. per square centimeter, for the first hour. The different plant forms considered are named at the left of the table and the room of the greenhouse in which each form was grown is indicated at the extreme right. The plant condition, as this became poorer with the drying-out of the soil, is shown for each time of observation, as follows: The soil values for condition 4 (vigorous growth) are in Roman type, those for condition 3 (some retardation) are in Italics, and those for condition 2 (beginning of definite injury), for condition 1 (very poor) and for condition 0 (vegetatively dead) are all in bold-face type. The values for condition 1 and 0 are distinguished from each other and from those for condition 2 by the introduction of a capital letter before each value, P for very poor (condition 1) and D for dead (condition 0). All values for plants in the vegetatively dead condition are separated from the rest of the table by a stepped line.

The soil area without plants (control) is represented in table II by the first line and the plant names are arranged in the order of the dates of onset of vegetative death, those at the bottom of the list representing plants that retained bright green and apparently healthy leaves longest after watering of the cultures had ceased. When the onset of vegetative death is recorded for several plant forms on the same day the plant names are arranged according to the decreasing order of magnitude of the corresponding soil-moisture indices. Tall oat-grass and orchard grass are recorded as having all their leaves dead on January 27, while Canada blue-grass did not reach that stage of withering till February 14 and sheep's fescue and red fescue did not reach it at all in the period of the experiment. The other forms take intermediate positions in the list.

It is apparent that there is a general tendency for the soil-moisture indices corresponding to the beginning of vegetative death to be larger in

TABLE II

INITIAL WATER-SUPPLYING POWER OF THE SOIL AND PLANT CONDITION AT END OF EACH 3-DAY PERIOD, DEC. 31, 1925, TO FEB. 14, 1926

DATA ARE EXPRESSED AS MILLIGRAMS OF WATER ABSORBED PER SOIL POINT, FOR THE 1-HOUR PERIOD AND FOR THE 6-CM. DEPTH

VALUES IN ROMAN TYPE CORRESPOND TO VIGOROUS PLANT CONDITION, THOSE IN ITALICS CORRESPOND TO PLANTS SOMEWHAT RETARDED IN GROWTH, WHILE INJURY TO THE PLANTS IS INDICATED BY BOLD-FACE TYPE

MARKED INJURY IS SHOWN BY THE PREFIXED LETTERS, P (VERY POOR CONDITION) AND D (VEGETATIVELY DEAD)

PLANT FORM	DEC. 31	JAN. 3	JAN. 6	JAN. 9	JAN. 12	JAN. 15	JAN. 18	JAN. 21	JAN. 24	JAN. 27	JAN. 30	FEB. 2	FEB. 5	FEB. 8	FEB. 11	FEB. 14	ROOM OF GREENHOUSE
Controls (no plants)	3003	2967	2784	2123	1396	749	486	259	135	75	56	49	40	42	34	28	
Tall oat-grass	3275	3005	2717	2040	*1365*	*475*	*261*	91	**P58**	**D52**	**D45**	**D40**	**D39**	**D49**	**D36**	**D28**	I
Orchard grass	3095	2991	2864	2240	*1219*	*630*	*197*	82	**P57**	**D42**	**D40**	**D42**	**D43**	**D43**	**D36**	**D33**	I
Brome grass	3095	2991	2864	2240	*1219*	*630*	*251*	94	60	**P49**	**D42**	**D38**	**D29**	**D39**	**D32**	**D26**	I
Rhode Island bent grass	2940	2729	2557	1875	1251	*963*	*360*	*142*	80	**P51**	**D40**	**D40**	**D37**	**D37**	**D32**	**D28**	I
Velvet bent grass	3140	3008	2922	2810	*2011*	*1110*	*908*	*225*	106	**P86**	**P48**	**D44**	**D37**	**D34**	**D31**	**D42**	I
Meadow fescue	2722	1889	1414	850	*243*	*133*	*93*	*75*	60	65	**P49**	**D43**	**D38**	**D42**	**D34**	**D26**	II
White clover	3003	2852	2540	1967	*934*	*453*	*157*	100	59	**P47**	**P44**	**D42**	**D38**	**D36**	**D33**	**D18**	III
Vermont bent grass	2722	1889	1414	850	*243*	*133*	*86*	*69*	55	54	**P42**	**P49**	**D40**	**D37**	**D35**	**D38**	II
Timothy	3245	3045	2890	2304	1896	*1097*	*579*	*360*	99	80	50	**P49**	**D37**	**D41**	**D36**	**D30**	I
Washington bent grass	3140	3008	2922	2810	2011	1110	*707*	*213*	61	55	**P48**	**P49**	**D36**	**D32**	**D38**	**D28**	I
Bermuda	2940	2729	2557	1875	1251	*963*	*466*	*265*	101	73	58	**P48**	**D34**	**D34**	**D36**	**D29**	I
Chewing's fescue	3166	2977	2590	1296	567	*299*	*223*	*117*	58	48	**P39**	**P39**	**D32**	**D34**	**D32**	**D39**	I
Alsike clover	3003	2852	2540	1967	*934*	*453*	*176*	*135*	85	59	**P51**	**P35**	**D30**	**D36**	**D33**	**D29**	III
Redtop	3245	3045	2890	2304	1896	*1097*	*656*	*325*	*81*	56	52	52	**P41**	**D37**	**D32**	**D28**	I
Kentucky blue grass	3022	2653	2222	1398	707	*495*	*395*	*329*	*165*	87	73	62	**P45**	**P41**	**D41**	**D23**	II
Perennial rye grass	3022	2653	2222	1398	*707*	*495*	*325*	*225*	100	66	55	**P55**	**P48**	**P48**	**D38**	**D35**	II
Canada blue grass	3022	2653	2222	1398	707	495	*399*	*293*	*133*	81	75	72	51	**P50**	**P41**	**D35**	II
Sheep's fescue	3275	3005	2717	2040	1365	*475*	*291*	*123*	*60*	48	36	**P40**	**P33**	**P31**	**P29**	**P27**	I
Red fescue	2722	1889	1414	850	243	133	*99*	*85*	*64*	52	47	41	32	**P33**	**P35**	**P29**	II

the upper part of the table and smaller in the lower part. Tall oat-grass showed all its leaves dead with a water-supplying power of 52, while red fescue and sheep's fescue had not reached that stage with supplying powers of 29 and 27 respectively. If the plant names are arranged according to the descending order of their soil-moisture indices when vegetative death was first noted, the list takes the form shown below, which is somewhat different from the list of table II, the index value being shown opposite the plant name in each case.

Tall oat-grass	52	Timothy Redtop	37
Velvet bent grass	44	Washington bent grass	36
Meadow fescue	43	Canada blue grass	35
Orchard grass Brome grass White clover	42	Bermuda grass	34
		Chewing's fescue	32
Kentucky blue grass	41	Alsike clover	30
Rhode Island bent grass Vermont bent grass	40	Red fescue	29
		Sheep's fescue	27
Perennial rye-grass	38		

Tall oat-grass is again shown as least resistant while sheep's fescue and red fescue are shown as most resistant. The difference between the values for adjacent forms in the intermediate region are, in many cases, probably not significant. It is notable that tall oat-grass stands by itself, having a much higher index value (52) than the next form on the list, velvet bent grass (44). The upper fourth of the total range of values (27–52) is represented by tall oat-grass alone. The lower fourth is represented by Chewing's fescue, alsike clover, red fescue and sheep's fescue.

Graphs

The data for the twenty soil areas were plotted in graphic form for convenient study and four of the resulting graphs (for the control area without plants and for the soils with tall oat-grass, with Kentucky blue-grass and with red fescue) are shown in figures 1–4, these being representative samples. The values of the water-supplying power of the soil for the several dates of observation form the ordinates. The abscissa for any ordinate represents the total accumulated evaporational water loss from the corresponding blackened atmometer sphere for the period beginning December 31 and ending at the time of the observation in question. Each graph thus represents primarily the rate of decrease in the soil-moisture value as related to the corresponding evaporation conditions and as related to the lapse of time. All four graphs are on the same scale. Dates of observation are shown above and the evaporation values below. The scale for soil moisture values is shown at the left. On the three plant graphs the health condition

of the plants is shown by the vertical subdivision of the area of the graph, each part bearing a notation that is self explanatory. The broken horizontal line near the base of each graph indicates the ordinate corresponding to the soil-moisture index value of 100, which has been emphasized as critical by WILSON (**32**).

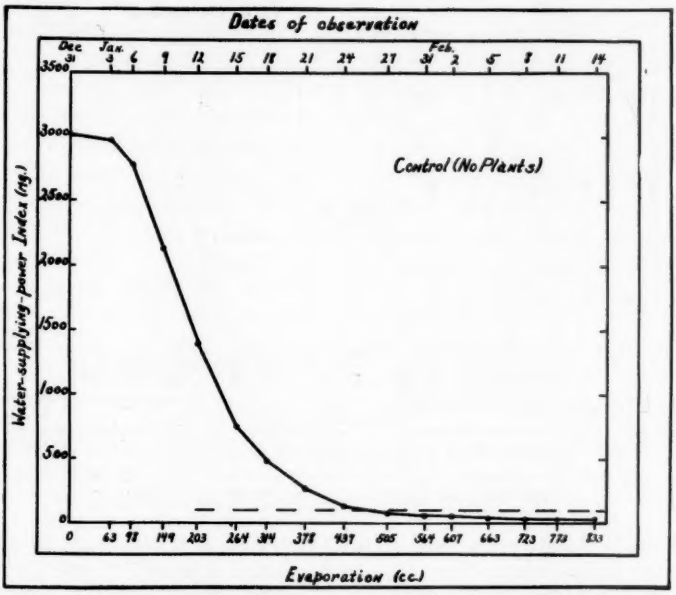

FIG. 1. Graph showing decrease in water-supplying power (ordinates) for 6-cm. depth in control soil mass, without plants, as related to lapse of time (abscissas, above) and cumulative evaporation from standard blackened atmometer sphere (abscissas, below), as soil dried out after final watering. Horizontal broken line represents supplying-power value of 100.

The average water-supplying power of the soil at the time of the first observation on December 31, twenty-four hours after the soil in the flats had been saturated with water, was in all cases about 3,000. This is markedly greater than the average value generally secured in WILSON'S (**32**) studies of a lawn soil out-of-doors after a hard rain, which is only about 2,000. Although the greenhouse flats were well drained, the height of their soil columns was very short in comparison with that for the open lawn, and the moisture content at the 6-cm. depth when drainage had just ceased was consequently much higher in the flats.

From this high value at the start the index of water-supplying power decreased as the soil dried out, the beginning of the rapid decrease being

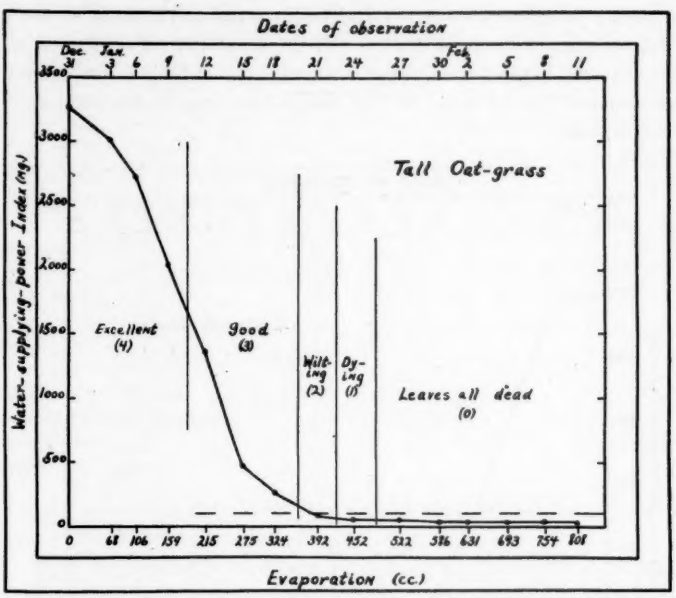

FIG. 2. Graph showing decrease in water-supplying power (ordinates) as in figure 1, but for soil with tall oat-grass. The area is divided into five parts representing five different conditions of health in the plants.

somewhat delayed in the case of the unplanted area but more prompt in the other cases, especially for the area planted with red fescue. For the ordinate range from about 3,000 to about 500 the graph approximates a straight line in each case; that is, the rate of decrease in the index value appears to have been approximately proportional to the accumulating total of evaporation from the blackened atmometer sphere. But since the evaporation total, measured at 3-day intervals, was almost proportional to the lapse of time, it is clear that the downward slope of the graph is also about proportional to the passage of time in the period of this most rapid decrease in the water-supplying power of the soil. With more frequent observations the question thus apparently raised (whether the graph slope in this region is to be considered as proportional to evaporation or to time) might have been answered, especially if the actual evaporation conditions had been less nearly the same for the three rooms. On *a priori* grounds it seems certain that the rate of evaporation, and of transpiration, rather than time itself should be regarded as the determining factor here. The slopes of these rectilinear parts of the four graphs are similar; the values of y/x being 12.3, 14.9, 15.0 and 13.3, respectively. The significance of the differences

among these values, if they are significant, cannot be judged from the data at hand.

The direction of each graph changes beyond the lower end of the straight-line portion just considered and more or less gradually approaches the horizontal. This means that the rate of decrease in the water-supplying power of the soil, in relation to evaporation from the atmometer (and to time), becomes slower as the soil dries out, which would of course be expected; the water-supplying power cannot decrease very much after low values have already been reached. The slope of the graphs is very low after the supplying-power value of 100 has been passed.

The water-supplying power of the planted soil areas generally decreased somewhat more rapidly than did that for the area without plants. This was probably due to the fact that the plants with their leaves in the air and their roots ramifying and advancing through the soil mass, increased the effect produced by the evaporation conditions in removing water from the soil and in the consequent lowering of the water-supplying power.

The idea already advanced by LIVINGSTON and KOKETSU (**17**), by LIVINGSTON and OHGA (**19**), by WILSON (**32**), and by WELTON (**31**), that the water-supplying-power value of about 100 (for the 6-cm. depth) may be regarded as critical for many plants and for ordinary evaporation condi-

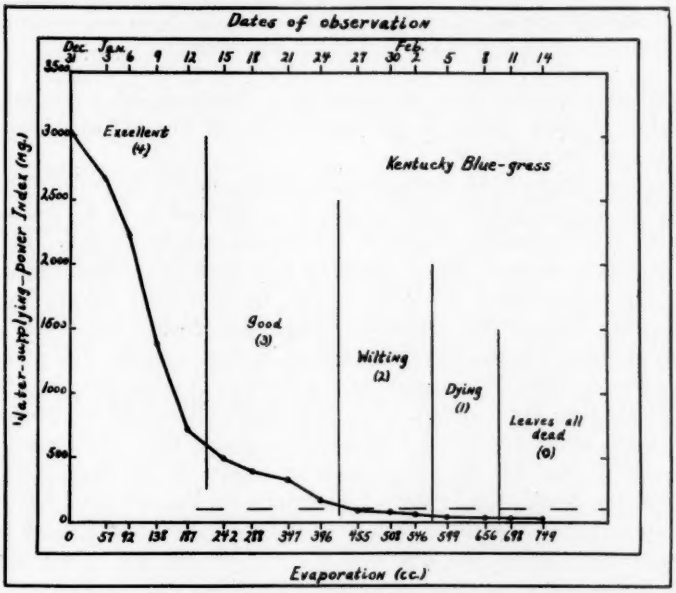

FIG. 3. Like figure 2, but for soil with Kentucky blue-grass.

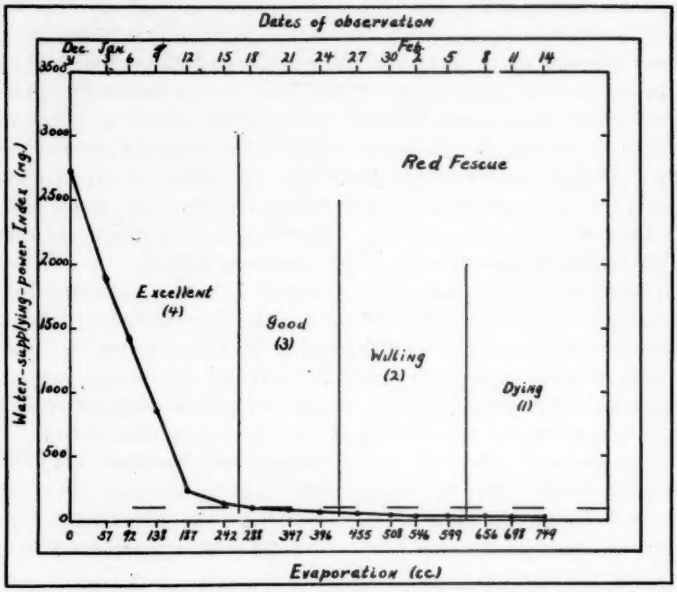

FIG. 4. Like figures 2 and 3, but for soil with red fescue. Note that all leaves were not yet dead at end of experiment.

tions, receives support from an inspection of figures 2 and 3 and table II. Several interesting points in this connection are brought out by an examination of the table.

There is only one case (that of red fescue) in which the index value for the water-supplying power of the soil was observed to be as low as 100 when the very beginning of growth retardation was first noted. In three cases (those of meadow fescue, Vermont bent grass and Washington bent grass) this critical value was first recorded six days after growth began to be retarded. Nine of the plants (tall oat-grass, orchard grass, brome grass, timothy, Bermuda grass, Chewing's fescue, redtop, Canada blue grass and sheep's fescue) showed the beginning of growth retardation nine days before the critical value was recorded. For four plants (Rhode Island bent grass, white clover, alsike clover and Kentucky blue grass) this period was twelve days. The period for the two remaining forms (velvet bent grass and perennial rye grass) was 15 days. Therefore, all but one (red fescue) of these nineteen plants began to show some slight effect of drouth much before the soil-moisture index had decreased to the value 100, but injury was not serious.

If we examine the table with reference to the soil-index values coincident with the beginning of definite injury (that is, with reference to the first boldface value given for each plant), we note that these values are below or about 100 for all forms. In the case of velvet bent grass serious injury was first observed with an index value of 106 and this is consequently not a significant exception to the general rule. In the case of meadow fescue, nothing worse than growth retardation was recorded for index values of 93 and 75, and definite injury first appeared only when the index value had decreased to 60. For sheep's fescue and red fescue the beginning of serious injury was recorded for index values of 48 and 52 respectively, while the beginning of growth retardation was shown for values of 475 and 99 respectively. All these fescues are well-known to be drouth-resistant. Their more wire-like leaves do not respond to drouth as promptly as do the ribbon-like leaves of most grasses.

The index value corresponding to pronounced injury (those marked P in the table) are all much less than 100, and the complete disappearance of bright green color (vegetative death) was first observed for values (marked D in the table) ranging from 52 to 35. The corresponding values for sheep's fescue and red fescue would have been somewhat below 27 and 29 respectively, but the experimental period was not prolonged sufficiently to bring about complete vegetative death in these two forms.

It therefore appears that a water-supplying power of 100 may be regarded as approximately and generally critical for these cultures. Had water been added so as to maintain the index value above 100 all of the plants would probably have continued to grow without very serious drouth injury. Nevertheless, it is clear that, while the value 100 appears to be a safe limit, below which the water-supplying power should not fall if vegetative health is to be maintained (for these plants and under these conditions of evaporation), yet the several plant forms differed markedly in their ability to withstand drouth.

A study of the graphs gives a clearer picture of the differences just considered than can easily be secured from the table. The vertical lines separating each graph (excepting that of the control area) into regions to show the five different growth conditions are each placed about the middle of the space representing an observation period, it being certain that the change in the plants from one condition to the next occurred between two consecutive observations. More frequent observations would, of course, have furnished data for the more accurate placing of these lines, but the error thus introduced can never be large. Supposing that each plant graph fairly represents the progress of its plants from excellent health to vegetative death, we may read from the graph the approximate water-supplying power of the soil for each of the points where the graph crosses the vertical

lines. These supplying-power values may be considered as approximately marking the transition from excellent to good growth, from good growth to wilting, from wilting to dying and from dying to complete vegetative death. At the same time we may note, in addition, the total amount of evaporation from the black atmometer that corresponds to the position of each vertical line, these totals being read along the base of the graph. Each represents the summation or integration of all the evaporation influences, from the time of the last watering to the time of the occurrence of the particular plant change in question. Table III presents the results of such a study of all the graphs, including the three here shown in figures 2 to 4.

Although the data of table III are to be considered as only approximately representative of the actual occurrences in these greenhouse cultures, yet they appear to show clearly that there were marked differences among the plant forms with respect to these critical values of the water supplying power of the soil at 6-cm. depth. With reference to any one of these criteria some forms appear to have been more drouth resistant than others and the order of drouth resistance is not the same for the several criteria. Velvet bent grass appears to have shown the first effect of drouth with an index of water-supplying power as high as 2421, but this value is probably erratic; the first criterion is not easy to employ. Allowing for such instances, however, the possibility seems to be indicated that a plant shown to be *highly* drouth resistant with respect to the onset of vegetative death may be shown as of *low* drouth resistance with respect to the change from excellent to good growth or with reference to the onset of wilting. For example, sheep's fescue, which appears to be very drouth resistant on the basis of onset of vegetative death, is like Washington bent grass (920) with respect to the change from excellent growth to good growth, while the last-named form is not shown to be especially resistant by the criterion of vegetative death, 42.

It may well be that we have in such cases indication of a very important ecological and physiological principle; namely, that some plants may be highly sensitive to drouth conditions while they are still growing but exceptionally drouth resistant after growth has been stopped or after wilting has supervened through the influence of inadequate water relations. In comparing different plants with respect to their different degrees of drouth resistance, it is necessary to take into consideration the physiological criteria employed, as well as the kind of externally effective influences dealt with. Our sheep's fescue was apparently not very drouth resistant toward soil-moisture conditions while it was still growing, but it was exceptionally resistant after growth had ceased and many of its leaves had died.

FOLLET-SMITH (5) in a summary of the water-supplying power of soils at the time of plant wilting, as ascertained by different investigators, found

TABLE III

APPROXIMATE CRITICAL VALUES OF THE WATER-SUPPLYING POWER OF THE SOIL AND OF TOTAL EVAPORATION FROM THE BLACK ATMOMETER (SINCE THE LAST WATERING), CORRESPONDING TO CHANGES IN THE CONDITION OF THE PLANTS DERIVED FROM GRAPHS FOR THE DATA GIVEN IN TABLES I AND II

NAME OF PLANT	FOR CHANGE FROM							
	EXCELLENT TO GOOD GROWTH		GOOD GROWTH TO WILTING		WILTING TO DYING		DYING TO VEGETATIVE DEATH	
	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION	SOIL INDEX	TOTAL EVAPORATION
	mg.	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*	*cc.*
Tall oat-grass	1700	186	178	358	75	422	55	487
Orchard grass	1730	186	142	358	70	422	50	487
Brome grass	1733	186	175	258	55	487	45	554
Rhode Island bent grass	1100	245	108	422	66	487	44	554
Velvet bent grass	2420	186	170	422	96	487	46	608
Meadow fescue	560	163	68	372	57	481	46	527
White clover	1470	176	130	346	53	471	43	585
Vermont bent grass	560	163	62	372	48	481	44	572
Timothy	1500	245	230	422	50	608	43	662
Washington bent grass	920	300	138	422	51	554	42	662
Bermuda grass	1090	245	92	487	53	610	41	662
Chewing's fescue	435	245	90	422	44	554	35	662
Alsike clover	1440	176	110	408	55	535	32	635
Redtop	1520	245	70	487	46	662	39	723
Kentucky blue grass	590	215	120	426	50	573	41	677
Perennial rye grass	1050	163	160	372	55	527	43	677
Canada blue grass	445	265	106	426	50	627	38	723
Sheep's fescue	920	245	54	487	38	508	.....	.....
Red fescue	112	265	56	426	32	527	.....	.....
Average	1121	216	119	405	55	527	43–	615+

this value to vary from 7.5 to 42, depending on the plants concerned and environmental conditions involved. The average value for this condition for the plant forms included in our table III was 55 and the value for a stage intermediate between wilting and vegetative death is 43. Our greenhouse values generally agree very well with those of FOLLET-SMITH, although our averages are somewhat higher. Many of the individual values fall near the 42-mg. value.

The evaporation data given in table III indicate in a general way, as would be expected, that these evaporation totals increase as the indices of water-supplying power decrease. The averages shown at the bottom of the table are instructive in this connection. Of course, they represent all the plants taken together. The suggestion that evaporation totals accumulated since the last watering may be employed as rough indices of the water-supplying power of the soil, has been implied by many writers in ecology and it has been considered by WILSON (**32**) in some detail.

Another method for securing indices of drouth resistance as related to evaporation may consist in determining from the graph for each plant form the total amount of water lost from the blackened atmometer between the time when the water-supplying-power index first fell below 100 and the time when the plants passed into the condition of vegetative death. This amount of evaporation is represented by the horizontal distance measured on the 100-line, from the intersection of the graph with that line to the intersection of the same line with the vertical line that separates sections 1 and 0. These intercepts might be calculated directly from the numerical data but the graphical method is simpler and sufficiently precise. Their values for the 19 plants are listed below in the increasing order of their magnitudes. They are expressed as cubic centimeters of evaporation (corrected) from the Livingston standard blackened atmometer sphere.

Tall oat-grass	100	Kentucky blue-grass	237
Orchard grass	107	Meadow fescue	249
Rhode Island bent grass	124	Chewing's fescue	252
Velvet bent grass	138	Redtop	275
Brome grass	168	Perennial rye-grass	281
Washington bent grass	178	Canada blue-grass	290
White clover	208	Vermont bent grass	300
Bermuda grass	209	Sheep's fescue	458+
Alsike clover	210	Red fescue	465+
Timothy	212		

The values for sheep's fescue and red fescue must be greater than 458 and 465, respectively, which are the largest of our series.

Tall oat-grass is again shown as least drouth resistant and sheep's fescue and red fescue are again shown as most drouth resistant. It is interesting

to note that very high drouth resistance was indicated for red fescue in the lawn studies reported by WILSON (**32**).

There seems to be no value of the water-supplying power that can correspond to "permanent wilting" for all these plant forms, although all had the same kind of soil and approximately the same evaporation and light conditions. This is, of course, just what would be expected from the physics of the soil-plant-air system and from the experimental studies on wilting points and soil moisture contents carried out by CALDWELL (**4**) and by SHIVE and LIVINGSTON (**26**). It becomes increasingly clear that the doctrine of unavailable soil moisture and wilting points, as set forth by BRIGGS and SHANTZ (**3**) and frequently accepted without question, is greatly in need of fundamental limitations based on still further critical study. VEIHMEYER and HENDRICKSON'S recent studies (**29**) are important in this connection.

The apparently rather definite and significant relation between the decrease in the water-supplying-power of the soil and the corresponding increase in the total amount of water lost from the blackened atmometer sphere since the last watering, is consistent, in general, with a similar relation emphasized by WILSON from his studies on lawn plants in the open. He found that the index of the water-supplying power of his lawn soil (which has somewhat higher water-holding power than the one used in these greenhouse cultures) was about 2,000 shortly after a rain and decreased by about 4.6 for each atmometric increment of 1 cc., for the range of water-supplying power from about 2,000 to 100. It is interesting to secure corresponding ratio values for these greenhouse cultures, for comparison. The average period dealt with in WILSON'S outdoor study was only 11 days in length and some rain occurred within the period in each case. In this greenhouse study, on the other hand, there was no irrigation or any addition of water to the soil during the period while the water-supplying power was decreasing from about 2,000 to about 100. Besides the unplanted area in the third greenhouse room, three planted areas, one from each greenhouse section, may be considered as representative; namely, those with alsike clover (room III), Kentucky blue grass (room II), and velvet bent grass (room I). The lengths of the periods in question for these four cases were 15, 21 and 15 days, respectively, for the planted areas, and 18 days for the unplanted area, the average length of period being 17 days. It will be noted that this period was considerably longer for the greenhouse cultures in winter than for the open lawn in summer, in spite of the fact that the period of the latter was not wholly without rain.

The supplying-power index of the alsike area was 1,947 on January 9 and it had fallen to 85 fifteen days later, on January 24—a decrease of 1,882. For this period the total loss from the blackened atmometer sphere in the same greenhouse room was 339 cc., and the index value consequently

decreased at the average rate of 5.55 for each cubic centimeter of total evaporation. The area with Kentucky blue grass had a water-supplying power of 2,222 on January 6 and on January 27 this had fallen to 87, the total decrease being 2,135 in 21 days. This period showed an accumulated water loss from the blackened atmometer sphere of 398 cc. and the average decrease in the index value was 5.36 per cubic centimeter of total evaporation. The water-supplying power of the area planted to velvet bent grass was 2,011 on January 12 and on January 27 it was 86, the decrease being 1,925 in this case. The evaporation for the period of 15 days was 363 cc. and the average decrease in the supplying-power index was 5.30 per cubic centimeter of total evaporation. For these three planted areas which do not differ significantly in this respect, the average rate of decrease in the index value is 5.4, for the range from about 2,000 to about 100—more accurately to about 80.

The unplanted area had a water-supplying power of 2,123 on January 9, which decreased to 75 by January 27, thus showing a decrease of 2,048 in 18 days, and the corresponding total evaporation during this period was 407 cc. This gives a decrease in the soil-moisture index of 5.0 per cubic centimeter of total evaporation. Thus the mean rate of decrease in the water-supplying power for the range considered and for the 6-cm. depth, was somewhat less for the unplanted area than for any of these planted areas, as would be expected; since no water was lost from the soil of the former through plant absorption and transpiration. The difference between the average rate for the unplanted area and that for the three planted areas is only 4 in 54, or 7.4 per cent. This is a small difference but it may represent the influence of this kind of vegetation cover on the drying-out of the soil at the 6-cm. depth, under the climatic and soil condition of these greenhouse cultures. These mean decrements of water-supplying power for each cubic centimeter of corrected water loss from the blackened atmometer sphere may be summarized as follows: For the open lawn in summer WILSON (**32**), 4.6; for planted greenhouse flats in winter, 5.4; and for the unplanted greenhouse flat in winter, 5.0.

The rate of decrease in water-supplying power per cubic centimeter of evaporation from the standard blackened atmometer sphere was greater in all four cases in the greenhouse than in any case recorded by WILSON for the open lawn. This was to be expected, since no addition of water to the soil occurred in the greenhouse, while there were light rains during every period of drying-out in WILSON'S study. Other differences may also be important, especially the difference between the two soils and between the components of the evaporation complex in the two studies, but both results are to be considered as only approximate.

From these and other similar considerations it seems possible that atmometer records might be employed in place of soil-point readings, to indi-

cate the need of irrigation and the approach of injury from inadequate water supply during drouth periods, at least for such plants as are dealt with in this study. For example, for a soil similar to those here considered it might be presumed that about 300 or 350 cc. of water might be lost from a properly exposed standard blackened atmometer sphere between two consecutive applications of water, each application being sufficient to wet the soil to its maximum field capacity. Atmometer records would be kept by cumulative daily readings and a thorough watering would occur on the day following the one on which the total of all readings (since the last watering) amounted to 350 cc. Then the summing would be begun again, etc. Such a procedure would be comparatively simple and it should maintain the water-supplying power of the soil at a higher value for greater depths. At the same time there would be little chance of deficient soil aeration through too long continued high water contents of the soil. This suggestion has not yet been tested in practice, but it may have real practical value for those who desire to maintain healthy plant growth, as for a lawn throughout the growing season, with a minimum application of water and without any periods of considerable growth retardation or discoloration due to drouth. The summation value to be employed would doubtless vary with the nature of the soil used and it might be much greater for specially drouth-resistant grasses, such as red fescue and sheep's fescue, than for such forms as velvet bent grass or Washington bent grass. It might be safe to neglect rain unless it was quite certain that any period of precipitation had brought the soil-moisture index to 2,000 or above.

This new suggestion, that atmometers might be employed to furnish a possibly valuable guide to the moisture condition of the soil, without recourse to any soil measurements at all, is worthy of serious attention in tests at the hands of ecologists and agriculturists, agricultural climatologists and others interested in the ecological aspect of plant water relations. The suggestion appears to be specially promising for stations where little or no rain falls during the growing season and where water must be supplied to cultivated plants by means of irrigation, but the effect of precipitation, when it occurs, may be taken into account after some preliminary study. The critical value of total evaporation would, of course, vary with the kind of plants grown, with their stage or phase of development, with the water-holding power of the soil, with its depth and the depth to which the plant roots penetrate, etc. The principle involved may be stated as follows: For any given upland plant at any stage of its growth and for the particular soil in which it is rooted, the water-supplying power of the soil mass occupied by the root system should be adequate for healthy growth just after the soil has been thoroughly wetted by rain or irrigation and allowed to drain, and the water-supplying power should remain adequate

for healthy growth until the continuously summed drying effect of the air and radiation conditions (as these operate to remove water from the soil by direct evaporation and by plant absorption and transpiration) has attained a certain critical value. It seems likely that the continuous summation of daily readings from a suitable form of atmometer suitably exposed may give a satisfactory measure of the summed drying influences and that such an atmometric summation may indicate approximately when the water-supplying power of the soil approaches a point where it would be inadequate for satisfactory growth of the plant.

Pot cultures

A few tests with potted broad-leaved plants were made in connection with the experiments already described. These will now be presented briefly. Ordinary 6-in. pots were used. The plant forms employed were buckwheat (*Fagopyrum esculentum* Gaertn.), kidney bean (*Phaseolus vulgaris* L.), *Coleus blumei* Benth., hollyhock (*Malva sylvestris* L.), and sensitive plant (*Mimosa pudica* L.). The cultures were started late in September, 1925, all with a mixture of sand, loam and humus like that used for the cultures in flats. Fifteen pots were prepared for each kind of plant, with five plants in each pot for buckwheat, four for bean, and one for each of the other forms. Some additional pots of buckwheat had fifteen plants each. The pots were watered at intervals until vigorous plants were secured. The last watering for buckwheat, bean and Coleus occurred on November 16, buckwheat and bean being then in bloom, and watering was discontinued April 9 for hollyhock and sensitive plant. The cultures were allowed to dry out gradually after watering had ceased, and soil-moisture determinations were made at the beginning of visible wilting and at two subsequent stages of drouth injury. The numerical results were recorded in terms of water content of the soil per unit of natural volume, as well as in terms of the water-supplying-power value determined by soil points for the 6-cm. depth and for the 1-hr. period. To determine the water content three cork-borer samples, 1 cm. in diameter and 7 cm. in depth, were taken for each test and these three were combined to form a composite sample. The resulting water-content value represents an average for the pot. Similarly, three soil points were used in each pot at each observation, the average reading being recorded. It should be noted that evaporation from the pot walls as well as from the soil surface accelerated the drying-out of the soil, and wilting occurred sooner than would have been the case had the containers been impervious to water.

The last two columns of table IV present the results of these tests when the plants were just beginning to wilt, when they were badly wilted and when the leaves had just become thoroughly dried. (What is here termed

"bad wilting" may be considered as approximately the stage called the beginning of permanent wilting by BRIGGS and SHANTZ, though no recovery tests were carried out on these pot cultures excepting for some of the pots of bean.) At the bottom of the table are given corresponding data for Coleus and hollyhock when in good condition, shortly before these plants began to wilt. The number of plants in a pot and the number of pots tested in each case are also shown in the table.

TABLE IV

SOIL-MOISTURE DATA FOR POTTED PLANTS IN THE GREENHOUSE

CONDITION OF PLANTS	PLANT NAME	NO. OF PLANTS IN POT	NO. OF POTS TESTED	WATER-SUPPLYING POWER OF SOIL AT 6-CM. DEPTH	AV. WATER CONTENT IN 100 CC. OF SOIL
Plants just beginning to wilt	Buckwheat	15	3	50	5.46
	Buckwheat	5	3	48	6.08
	Bean	4	3	42	5.16
	Coleus	1	3	40	5.47
	Hollyhock	1	4	43	5.36
	Sensitive plant	1	8	44	6.48
Plants badly wilted	Buckwheat	5	6	36	4.60
	Bean	4	6	34	5.05
	Coleus	1	3	36	5.47
	Hollyhock	1	4	32	4.16
	Sensitive plant	1	3	23	3.88
Leaves dried up	Buckwheat	5	3	27	4.03
	Bean	4	3	26	4.25
	Coleus	1	3	29	4.20
Plants in good condition	Coleus	1	3	164	8.26
	Hollyhock	1	3	60	7.73

Since the observation of the second and third stages of drouth injury here dealt with was not so definite as that of the beginning of wilting, the most interesting values to study are those of the first section of table IV. In buckwheat, bean and Coleus wilting begins as a noticeable drooping of the leaf blades. In hollyhock the first sign of wilting is a flattening of the blade, previously somewhat concave upwards. The ultimate leaflets of the sensitive plant fold at the beginning of wilting as they do in assuming their night position or as a result of mechanical stimulation. The use of this definite response has been emphasized by KOKETSU (**10**) as a criterion for fixing upon an exceptionally precise stage of wilting in this plant.

For all these plants the beginning of wilting occurred with a water-supplying power between 50 and 40 and with a water content between 5.15 and 6.48 per cent. (on the basis of natural volume). From the data for water-supplying power it appears that the buckwheat plants required a significantly higher value (48 or 50) to prevent the onset of wilting than was required by the other forms and that the latter were so nearly alike with respect to the water-supplying power of the soil at the beginning of wilting that the differences indicated (between 40 and 44) may be considered as insignificant. The value for the sensitive plant (44) is probably somewhat more reliable than the values for bean, Coleus and hollyhock, because of the larger number of tests involved in the average for the sensitive plant as well as because the beginning of wilting was probably more precisely observed in that case than in the others. Whether fifteen or five plants of buckwheat were present in the 6-in. pot made no significant difference in the soil-point value corresponding to the beginning of wilting. It may be noted that the soil point readings at the time of bad wilting for the five species listed in the second section of the table all fall within the limits of 7.5 to 42 mg. mentioned by FOLLET-SMITH (5) in his summary of the wilting point values obtained by various workers. Nevertheless, the soil-point values given for successive stages of drouth injury for each of the other forms are remarkably consistent and indicate such differences as might be expected. The water-content data are not so consistent, however.

It appears in general that both water-supplying power and water content are to be regarded as useful for studying changes in soil-moisture conditions between two successive stages of drouth injury, but that the soil-point method is apt to be somewhat more sensitive and probably more precise. The soil-point method gives promise of great advantages when the soils of a series are not nearly alike, but those advantages are not shown in this series because the same kind of soil was used for all cultures.

From the data of table IV, buckwheat appears to have shown about the same degree of drouth resistance as was shown by bean and Coleus if we use the second or third stage of injury, while it was less resistant than the other two forms if we use the first stage of injury. Bean, Coleus, hollyhock and sensitive plant appear to have shown about the same degree of drouth resistance on the basis of the beginning of wilting but the sensitive plant showed a much higher degree of drouth resistance than any of the others on the basis of the onset of bad wilting. Such differences between the indications secured for different degrees or stages of water deficit may be partly due to uncertainty in observing the exact stage desired in any instance, but it seems reasonable to suppose that different stages of wilting may not agree in their indications as to drouth resistance, as has been said in the earlier part of this paper.

The results secured from these pot tests cannot be compared in detail with those from the cultures in flats, described in the first part of this paper, notably because the period of drying-out of the soil mass was much shorter in the case of the pot tests. Evaporation conditions for the two series were somewhat different also, and these should influence the results. In a general way, the two series agree in showing the remarkable sensitiveness of the soil points when compared with the plants and in furnishing evidence of the promise and practicability of the soil-point method for studying soil-moisture conditions.

Conclusion and summary

The water-supplying power of the soil adjacent to plant roots is the single dynamic soil feature that directly affects plant water relations. All other soil features or characteristics that appear to influence plant activity through water relations are indirect in their operation, for they either affect the water-supplying power of the soil (directly or indirectly) or else they affect the aerial environmental complex or the capacity of the plant itself to absorb or conduct or retain water. The water-supplying power of a soil may be directly measured by the soil-point method, without reference to the kind of soil used, its degree of packing or its water content. The numerical values or indices secured represent the ability of the soil to deliver water to an absorbing surface. The standard instrument has an absorbing area of 12 sq. cm. and the standard period of its exposure in the soil is 1 hour. If any index value is divided by 12 the resulting quotient is the hourly rate of water delivery by soil to soil point across an area of 1 sq. cm., for the hour of the test.

This study of greenhouse cultures in winter (carried out at the Johns Hopkins Laboratory of Plant Physiology, in Baltimore) deals with some of the more easily detected relations that exist between (a) soil-moisture conditions as represented by the water-supplying power of the soil at a depth of 6 cm. and (b) plant health and vigor as these alter with the drying-out of the soil mass after the cessation of watering. As this drying-out progresses the plants first show growth retardation, followed by complete cessation of growth. They then wilt and finally die by degrees, the leaves being, of course, the first parts to be visibly affected.

For any particular soil mass the water-supplying power at any particular place in the mass is specific for any given instant. It is greater the greater is the water content and it is different for different degrees of packing. An upland soil with water content equal to its maximum field capacity (as when downward flow of water has just ceased after a heavy rain or after the artificial application of water) can supply water to an absorbing surface, such as that of root, root-hair or soil point, at a rapid rate; but the supplying power steadily decreases as the soil dries out through absorption

of water by plant roots and through evaporation into the air above. The water films become thinner, the resistance to capillary movement increases and the capacity of the soil to supply water to a root system eventually becomes insufficient to maintain health in a plant with stationary root surfaces. However, plants generally extend their root systems continually into new soil regions, where the water content and water-supplying power have been less depleted. When the combined effects of root advance and capillary movement of water fail to supply water at a rate sufficient to make up for the loss and use of water by the plant (transpiration, tissue enlargement, etc.), then the water content of the plant begins to decrease and wilting soon begins. If no water is added to the soil adjacent to the root system, wilting becomes more pronounced and extensive and withering and death eventually supervene. In the meantime, the water-supplying power of the soil region occupied by the root system continues to decrease. Although it may be true that the production of root branches and root-hairs is generally, or, in some cases, most vigorous in soils with supplying powers somewhat below the optimum for most active top growth, nevertheless pronounced wilting and withering of the leaves is probably accompanied by great retardation or cessation of root growth. For any short interval in the drying-out period there appears to be a fairly definite relation between the health of the plant and the current magnitude of the water-supplying power of the soil about its roots, if we consider other influential conditions as remaining practically unchanged.

This paper deals mainly with (a) the progressive decrease in the initial water-supplying power of the soil (measured by means of soil points applied for one hour at the 6-cm. depth) as the soil dries out after watering has been discontinued, and with (b) the accompanying gradual change in the condition of the plants as they pass from excellent to good health, from good health to the beginning of wilting, from the onset of wilting to the occurrence of bad wilting, and from the onset of bad wilting to the onset of vegetative death, (when all leaves are withered). The occurrence of "bad wilting" in our cultures corresponds closely to the onset of "permanent wilting" as roughly defined by BRIGGS and SHANTZ.

In the main series of experiments, greenhouse cultures in wooden boxes or flats were employed. There were seventeen different grasses and two clovers, also three control soil areas without plants. The same kind of soil was used for all flats, a light loam. When a well-established turf had been formed in all cultures the soil was thoroughly wetted for the last time and watering was discontinued. Throughout the ensuing period of the drying-out of the cultures records of plant condition and of the water-supplying power of the soil were made at frequent intervals and corresponding records were kept of air temperature and of evaporation and water loss from

standardized LIVINGSTON spherical atmometers, white and blackened with lampblack. The indices of water-supplying power are expressed in terms of milligrams of water absorbed by a single standard soil point (about 12 sq. cm. of absorbing surface) in one hour, at a depth of 6 cm. beneath the top of the soil mass.

The numerical data are given in tables, and sample graphs are presented for the control soil areas without plants and for the cultures of tall oat-grass, Kentucky blue-grass and red fescue. The numerical value for the water-supplying power of the soil at the 6-cm. depth decreased from about 3,000 to about 500 in nearly the same manner in all the flats. After the first few days the rate of decrease was nearly uniform for a long period and about proportional to the lapse of time. When the index value had reached about 500 the rate of decrease became slower, being very slow for values below 100.

In twelve out of the nineteen plant forms studied actual drouth injury became apparent about the time that the decreasing water-supplying power of the soil attained the critical value of 100. For the remaining seven forms injury did not appear until still lower values had been reached.

Of these nineteen species, tall oat-grass showed the lowest degree of drouth resistance; its leaves were all dead by the time the index of the water-supplying power of the soil had decreased to 52. Red fescue and sheep's fescue showed the highest resistance; they still had many living leaves when the index value had decreased to below 30. At the end of the experiment period of about six weeks, with no water added to the soil and with a total evaporation of about 800 cc. for the period (from the blackened atmometer sphere), all of the leaves were dead for all forms except sheep's fescue and red fescue.

Clear relations were shown between the rate of decrease in the water-supplying power of the soil and the corresponding rate of water loss from the blackened atmometer. For the period during which the value of the water-supplying power was decreasing from about 2,000 to about 100 this relation was about the same for all soil areas; the index value was diminished, on the average, by 5.4 (in the case of the planted flats) or by 5.0 (in the case of the control area) for each cubic centimeter of total evaporation. These ratio values are somewhat greater than a corresponding value (4.6) found by WILSON in his studies on the soil of an open lawn in summer at Baltimore.

It is suggested that the total evaporation that has occurred from a suitably exposed standard atmometer since the last preceding considerable application of water to the soil may offer a means of approximating the water-supplying power of a field soil at any time within a rainless or nearly

rainless period. If the losses from the atmometer are summed from day to day, as such a drouth period advances, it appears from our studies and from that of WILSON that the summation amounts to about 350 cc. or 400 cc. at the time such plants as lawn grasses begin to show drouth effects. The critical evaporation total should depend on the kind of soil, kinds of plants, etc., and upon the daily rate of evaporation. It should be different for sufficiently different climatic complexes.

The several cultures did not indicate a uniform relation between amounts of total evaporation (since the last watering) and the onset of vegetative death in the plants. Beginning when the soil-moisture index was about 100, vegetative death occurred for tall oat-grass when only 100 cc. of water had been lost from the blackened atmometer sphere. For Canada blue-grass this evaporation total was 290, while for sheep's fescue and red fescue vegetative death had not occurred with evaporation totals of 458 and 465, respectively.

A few experiments were performed with pot cultures (in ordinary 6-in. pots in the greenhouse, in winter and spring) of buckwheat, Coleus, sensitive plant, bean and hollyhock. These were treated about like the cultures in flats, having the same kind of soil as those, but records were made of volumetric soil-moisture content as well as the soil-point readings, at intervals during the drying-out of the soil following the last watering. These two methods for securing numerical indices of soil-moisture conditions generally agreed very closely. Since the soils were alike for all cultures the two methods should be about equally useful in such a series, but the dynamic method appears to be somewhat more precise.

The beginning of wilting occurred, for all five of the potted forms, with supplying-power values between 50 and 40, and with volumetric residual water contents of the soil between 6.48 and 5.15 per cent. Buckwheat appeared to be least drouth-resistant by these criteria and the other plants were apparently somewhat more resistant and about alike. Hollyhock and sensitive plant were found to be specially suitable for studies of this type, because of their delicate and easily observed leaf responses at the beginning of wilting.

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AVAILABLE WATER AND THE WILTING OF PLANTS

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Soil moisture in relation to plant growth is expressed upon the basis of its availability to the plant. Botanical or agricultural papers in which comparisons are made of the effects of different soils and soil moistures upon the growth of plants must necessarily consider the ability of the plant to obtain the moisture present in the particular soil. It is apparent however to those who have given such problems some study that our methods of determining the availability of water in a given soil are not very accurate nor very easily applied. It was with the view in mind of studying the plant in wilting and to improve, if possible, some of the methods used in investigating the water relations at wilting that this work was undertaken.

SACHS (**9**) as early as 1859 demonstrated the importance of the textural properties of the soil in determining the percentage of soil moisture which is available to plants. BRIGGS and SHANTZ (**1, 2, 3**) designated that percentage of moisture which remains in the soil at permanent wilting as the wilting coefficient. Permanent wilting was defined as the stage of wilting of a plant from which it fails to revive within 24 hours if placed in a saturated atmosphere. From their results they concluded that the wilting coefficient varies with the character of the soil but is very nearly the same for all types of plants. The differences found when different species were used were interpreted as being due to variations in root distribution. They were led to conclude further that the soil moistures at which plants are grown have no effect upon the value of the wilting coefficient.

VEIHMEYER and HENDRICKSON (**14**) have found evidence that plants are able to reduce the moisture content of different soils to different degrees of "dryness" before permanent wilting sets in. Therefore they believe that the wilting coefficient cannot be calculated from the moisture equivalent as is commonly done.

The soil has a water-retaining power at the wilting coefficient of 3 to 4 atm. according to SHULL's (**10**) calculations on the basis of water intake of cocklebur seeds. The average osmotic pressure of the plant root is equivalent to 7 or 8 atm. as determined by HANNIG (**5**). However according to URSPRUNG (**12**) the average effective osmotic pressure is more likely to fall to 3 or 4 atm. Even with unimpaired ability of the root to absorb moisture the practical cessation of movement of water in the soil at low moisture ranges would cut down water intake to an entirely inadequate rate.

URSPRUNG and BLUM (**13**) have shown that the effective osmotic pressure of roots may vary by several atmospheres in a relatively short time. For

example when *Vicia faba* was transferred from tap water to a 0.2N solution of sucrose and with the removal of the greater part of the root system, the remaining roots increased in effective osmotic pressure from 5.3 to 6.3 atm. RENNER (**7**) found that *Phaseolus* seedlings previously adapted to weak KNO_3 solution were able to absorb water from 3–5 per cent. KNO_3 solutions which represent 12–20 atm. of pressure respectively. RICOME (**8**) was able to cause the rapid wilting of *Vicia faba* and *Ricinus* plants when transferred from Knop's solution to Knop's solution with 1 per cent. NaCl.

Comparatively few investigators have studied the *plant* at permanent wilting. CALDWELL (**4**) found a decrease of 30 per cent. in the water content of the leaves of plants at permanent wilting. The percentage of water in leaves at permanent wilting was approximately constant for all individuals of the same species at the same stage and degree of development. This constant was unaffected by external conditions under which the wilting occurred. The water of the stem tissue was not considered, since according to this author, the water of the leaves of six weeks' old *Phaseolus* plants is much greater proportionally than that found in the stems. KOKETSU (**6**) found this constant (*i.e.*, the water present in the leaves at permanent wilting) to be affected by the character of the soil. With *Glycine* it was found that the higher the water-holding capacity of the soil the larger the critical water content of the leaves. Opposite results were obtained with *Coleus*. The ratio of the critical water content at permanent wilting to the water content at full turgidity was found to be higher for *Coleus* than for *Glycine* or *Mimosa*. According to KOKETSU, the lower one finds this ratio to be, the more xeric the plant.

Materials and methods

Seedlings of *Phaseolus vulgaris* (Burpee's Stringless Greenpod) as grown under greenhouse conditions were used in all the experiments. In most cases the plants were grown in brown silt loam soil. In a few instances sand cultures (silica sand) were used. The calculated wilting coefficient for the sand was 0.213 per cent. and that for the soil was 17.27 per cent.

In preliminary experiments direct determinations of the wilting coefficient of the soil were made according to the method of BRIGGS and SHANTZ (**1**). Plants were grown in soil about 1 inch deep, both with and without a wax seal. Each culture was brought up to its original weight and moisture content daily or every second day until the plants had reached the stage at which water was no longer applied. Soil samples were taken at permanent wilting from the deeper soil in which actively absorbing roots were located. All soil samples were dried to a constant weight at 100° C. and the per cent. of moisture calculated on the basis of the dry weight.

Other experiments were set up with the cultures growing in auto-irrigators in order to maintain a more nearly constant soil moisture. A description of this irrigator is to be published as a separate paper. There was space for nine soil containers in an irrigator; three of the containers were maintained at a soil moisture of approximately 15 per cent., three others were at approximately 26 per cent. and the remaining containers were held at a soil moisture of about 55 per cent. The soil moisture in a given container varied somewhat with different irrigators but was approximately constant throughout a single experiment. Six plants were grown in the soil of each container.

In starting a group of plants in an irrigator the seed was allowed to begin its germination in wet sand. It was then transferred to the soil, discarding at the same time all unsatisfactory seeds. The soil in all the containers of an irrigator was held at approximately 27 to 30 per cent. of moisture for three or four days or until the seedlings began to push through the soil. Soon thereafter the soil moistures changed to the percentages shown in the tables.

It will be noticed in several of the tables to follow that in certain cases the soil moistures at which the plants were grown were below the wilting coefficient. Due to the construction of the irrigator it was impossible to get a sample of soil which was representative of the moisture supply upon which the plants were depending except in those cases where the soil moisture was somewhat above the wilting coefficient.

In certain experiments the plants thus grown were subjected to permanent wilting and the per cent. of water present in the wilted plant determined. Permanent wilting was considered to be that state of wilting from which a plant will not regain the turgidity of its leaves if placed in the dark in a saturated atmosphere for a period of 24 hours. To determine the per cent. of water present in plant tissue, the material was collected in the fresh state in small closed containers, weighed, dried for 3 days at 100° C. and reweighed.

Plants grown at the different soil moistures provided by the system of automatic moisture control were used in tests in which the dry weight per unit of leaf area was measured. The leaf areas were determined with the aid of planimeter tracings of blue-printed or pencil-traced leaf margins.

In order to make the various tests more uniform the plants were not used until they had reached a definite stage of development. The stage most used was that in which the seedling had developed 2 well expanded, simple leaves (its first true leaves) and the internode above them was not over 5 mm. in length. The cotyledons at this stage were badly shrivelled but had not fallen off. In other plants the per cent. of water was deter-

mined when every leaflet of the first compound leaf was over 2.5 cm. in length.

To compare the wilting of plants grown in soil with that of plants grown in water cultures, a number of bean seedlings were grown in tap water in gallon earthenware jars. The water was changed daily in order to give more favorable conditions of growth. When the young plants had attained growth stages similar to those used in the tests just described, they were transferred to tumblers of tap water plus sodium chloride in various concentrations some of which were sufficient to produce wilting. The rate of wilting was increased in certain cases by means of air currents from an electric fan.

Results and conclusions

By reference to table I it is seen that direct determinations of the wilting coefficient gave values slightly lower than the values calculated from the moisture equivalent. This may have been due to certain properties of the soil used or to variations in the methods from those of Briggs and Shantz. A serious difficulty which is always apparent in the direct

TABLE I

Direct determinations of the wilting coefficient of silica sand and brown silt loam soil
Plants grown at the soil moistures indicated

Number of determinations	Original moisture content of soil	Moisture in soil at permanent wilting
	a. Results with silica sand	
		per cent.
3*	8% of the dry weight	0.047 ± 0.005
5*	11% " " " "	0.048 ± 0.004
5*	13% " " " "	0.115 ± 0.021
	b. Brown silt loam soil	
5†	32% of the dry weight	15.98 ± 0.11
7†	40% " " " "	17.20 ± 0.18

Wilting coefficient of sand calculated on the moisture equivalent – 0.213 per cent.
Wilting coefficient of soil calculated on the moisture equivalent – 17.27 per cent.
* Duplicated samples.
† Triplicated samples.

determination is the wide difference in the rate of wilting of individual plants growing in the same container.

Plants which were growing in sand could be wilted very rapidly by withholding water. In soil cultures the wilting was less rapid, being

an especially slow and gradual process when the soil surface was sealed over with wax. Seedlings growing in a soil culture which dries out very gradually develop a smaller leaf surface, a dark green color and gradually acquire more and more of the characteristics of xeric plants. It is highly probable that in this process the per cent. of bound water increases and perhaps there are cellular changes which are as yet little understood but which may be important from the standpoint of the wilting of the plant.

In both the sand and the soil cultures (table I), which were set up in shallow containers and watered at the surface from day to day to bring the culture up to the required moisture percentage, the containers in which a higher soil moisture was maintained previous to wilting, gave a higher wilting coefficient. The determinations were rather few but the tendency is in the direction which one would expect if a somewhat more xeric type of plant develops in a substrate maintained at a lower moisture content.

In order to study the variation in water present in a plant at permanent wilting a number of seedlings were grown under constant automatic water supply. In bringing the plants to permanent wilting they were treated in three different ways: (1) plants were wilted in the soil in which they grew by removing the container from the irrigator; (2) plants were removed from the soil without mutilation of the roots and allowed to dry on a wooden drying rack; (3) seedlings were cut at the surface of the soil and wilted upon the drying rack. The method by which the plants were brought to the state of permanent wilting had but little effect upon the water content of the plant when it reached that stage. However the rate of wilting was changed considerably according to the method used. The seedlings wilted very rapidly when they were removed from the soil with the root system left attached; they wilted less rapidly when cut at the soil, the cut surface having been waxed over, and finally the greatest time was required to wilt those plants which were left in the soil.

The data in table II, trial numbers 1–6 inclusive, indicate that, generally, the water held in the plant when it reaches the state of permanent wilting is greater if the plant has been growing at the higher percentages of soil moisture. This seems to occur in spite of the fact that the more xeric type of plant would tend to retain more bound water.

Wide variations in the rate of wilting of individual plants occurred throughout these tests. The differences were least evident in plants removed from the soil and wilted without removal of the root system. Where the seedlings were cut at the soil surface or in trials in which the plants were wilted without removal from the soil, different plants showed considerable variation in the rate at which they reached the state of permanent wilting. In certain cut plants the time required for wilting was 3 or 4 times the average. Uneven distribution of soil moisture might account for

TABLE II

MOISTURE CONTENT OF PLANTS AT PERMANENT WILTING

PLANTS WITH SIMPLE LEAVES			PLANTS WITH FIRST COMPOUND LEAF		
TRIAL NO.	SOIL MOISTURE	AVERAGE MOISTURE CONTENT*	TRIAL NO.	SOIL MOISTURE	AVERAGE MOISTURE CONTENT*
	per cent.	*per cent.*		*per cent.*	*per cent.*
	56	735		56	759
1	40	743	7	50	585
	20	704		49	688
				13	740
	42	997		53	724
2	19	865	8	46	628
	12	628		11	605
	52	1070		46	628
3	42	1026	9	43	633
	13	728		41	612
				17	620
	53	926			
4	28	829	10	54	655
	15	742		12	575
	49	943			
5	24	771	11	51	680
	13	731		44	581
	15	692			
6	15	696			
	10	639			

* Each percentage represents the average of from 6–9 determinations.

variations in the wilting rate of plants remaining in the soil but could only indirectly affect the rate in cut plants.

The data of table II illustrate the wide variation in the amount of water present in different sets of plants when brought to the state of permanent wilting. For example, the figures in trial 1 may be compared with those of trial 3, the two groups of plants having been grown under similar soil moisture conditions. The two upper soil moistures were sufficiently high to give an abundant supply of water to the plants and no great difference was apparent in leaf characteristics. Plants grown at the lowest soil moisture, that is, 10 to 20 per cent., were quite different from the others. One of the most important causes of the lack of consistency in the data lies in

the difficulty of judging the wilting, especially if the plants were brought to permanent wilting on different days.

The per cent. of water present in older plants at the stage of permanent wilting is shown in table II, trials 7–11. These plants had developed the first compound leaf before they were subjected to the conditions of wilting. The data given do not show a consistently lower water content at permanent wilting in the case of the plants grown at a lower soil moisture. The youngest compound leaf is always the last to wilt; it is also the most difficult to detect wilting in due to its size and the presence of a large number of epidermal hairs. Since in the seedling stage a new leaf is continually developing while the last one is enlarging, the determination of permanent wilting is interfered with.

The determination of the "end point" of permanent wilting is made more complex by the fact that single plants which had apparently reached permanent wilting would often revive as many as three or four times after short exposures to the saturated atmosphere of a moist chamber. In other words, in a dry atmosphere the losses of water from the leaves were too rapid to be covered by the movement of water from the regions of storage in the hypocotyl. Seedlings which were wilted without the removal of the roots from the soil and especially those which were cut at the soil surface were quite likely to regain the turgor of their leaves if placed in a moist chamber. The groups of plants which were removed from the soil without mutilation of the root system wilted more rapidly than did the plants given the other treatments; they were also less likely to revive in the moist chamber. The seedlings grown in a dry soil, in many cases failed to revive because of a low per cent. of stored water.

The degree of drooping which a leaf displays, such as is characteristic of permanent wilting, may be indicative of a fairly narrow range of water content in the tissues of the leaf blade but it does not tell much with regard to the per cent. of water present in other parts of the plant. The more nearly permanent the wilting becomes the closer is the approach to a constant and similar water content in all the organs of the plant. It is to be expected, therefore, that determinations of leaf moistures at the critical point of wilting would be more consistent than would determinations for the entire plant.

Analyses of different regions of the plant gave proof that the hypocotyl is an important water storing organ of the bean seedling. Uprooted plants were exposed to dry air for 0, 0.5, 1, 2, 3 and 4 hours, then cut into 5-cm. sections for the determination of the percentage of water present. Table III gives the water content of the root and of the proximal (number 1) and successive 5-cm. sections of the shoot. Corresponding sections from two or three plants were weighed together in the same weighing bottle. All of

TABLE III

MOISTURE DETERMINATIONS ON 5-CM. SECTIONS OF SEEDLINGS UPROOTED PLANTS EXPOSED TO AIR FOR PERIODS INDICATED PREVIOUS TO CUTTING INTO SECTIONS

PERIOD OF EXPOSURE	SECTION OF PLANT	AVERAGE MOISTURE CONTENT*	PERIOD OF EXPOSURE	SECTION OF PLANT	AVERAGE MOISTURE CONTENT*
		per cent.			*per cent.*
None	Roots	953	2 hours	Roots	167
	1	1168		1	961
	2	1018		2	1090
	3	963		3	836
	4	899		4	936
	5	963		5	681
	6	957			
30 min.	Roots	577	3 hours	Roots	140
	1	1205		1	999
	2	1085		2	1006
	3	1133		3	931
	4	703		4	1018
	5	798		5	802
1 hour	Roots	154	4 hours	Roots	122
	1	1184		1	1074
	2	948		2	913
	3	873		3	814
	4	795		4	.....
	5	638		5	326

* Each percentage represents the average of 3 determinations.

the plants used had begun to develop the first compound leaf. The effect of different periods of wilting upon uprooted plants is shown in the table. The check plants were weighed immediately upon removal from the sand culture; other plants were wilted for the periods shown.

Beginning at the lower end of the stem a decreasing gradient of the water present is apparent in successive regions up the stem. The plants which were wilted for two or for three hours give some evidence at least that in wilting the root draws upon water present in the hypocotyl. The very uppermost leaves are the last to wilt in spite of the fact that the upper part of the stem is constantly losing water very rapidly and usually shows a lower water content.

That the degree of wilting may not necessarily be a criterion of the moisture content of the plant is indicated as possible by the data in table IV. It is shown that the dry weight per unit of leaf area is distinctly

greater for plants grown in the drier soil. The difference is more significant in the case of the younger plants with only simple leaves. The greater dry weight per unit of area occurs with the development of numerous veins, numerous small cells with smaller vacuoles and less water. The increased amount of mechanical tissue supporting the leaf blade of the dry soil plant made necessary a greater loss of water to produce an equal drooping of the leaves. TUMANOV (**11**) found that leaves acquired a xeromorphic structure with more mechanical tissue present if the plants were subjected to alternate periods of optimal and sub-optimal soil moisture. The data in table IV explain in part the correlation between soil moisture and the quantity of water present at permanent wilting as given in table II. Thus the dry soil plants being slow to show signs of wilting have time to lose considerable stored water before they would be judged as permanently wilted.

TABLE IV

DRY WEIGHT OF LEAF TISSUE PER SQUARE CENTIMETER OF LEAF AREA

SOIL MOISTURE	NUMBER OF LEAVES	AVERAGE WEIGHT PER SQ. CM. LEAF AREA
per cent.	Simple leaves	$gm. \times 10^{-6}$
50	16	1628 ± 21.6
25	16	1685 ± 24.8
19	8	2128 ± 61.7
16	16	2196 ± 27.5
12	8	4333 ± 45.4
	Compound leaves	
42	7	1745 ± 35.9
22	4	1442 ± 44.2
11	2	2098 ± 28.8

The fact that the leafy top of the seedling depends upon stored water in the hypocotyl when the moisture supply from the soil is cut off would lead to the conclusion that the critical point is reached in the soil (*i.e.*, absorption by the root falls to a very low value) some time before wilting is evident. Absorption at a low rate is continuous whether the plant is depending upon stored water to maintain leaf turgidity or has become permanently wilted. Without a wax seal, evaporation may cause more depletion of the soil moisture and the final result may be that the wilting coefficient determined upon the basis of the permanent wilting of the leaves

may give a value somewhat lower than the true value for the critical state in the soil. Furthermore the plant with a xeromorphic leaf structure could use the soil moisture for a longer time before permanent wilting sets in than could a plant grown under more nearly optimal conditions of soil moisture. The results of the direct determinations of the wilting coefficient (table I) support this view.

For comparison with the soil cultures, plants were wilted in water cultures by transferring plants which had been growing in tap water to solutions of tap water plus various quantities of sodium chloride. Table V

TABLE V

DEGREE OF WILTING OF SEEDLINGS PLACED IN VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE SOLUTION. PLANTS WITH SIMPLE LEAVES

SODIUM CHLORIDE	TIME OF OBSERVATION				
	APRIL 4 10:30	APRIL 5 10:00	APRIL 6 9:00	APRIL 7 9:00	APRIL 8 11:00
Moles					
0.07	o	o	o	*	o
0.08	o	o	*	*	*
0.09	o	*	*	*	*
0.10	o	*	*	*	*
0.12	o	***	***	***	*
0.14	o	***	*	*d	dead
0.16	*	***	**	***	"
0.18	*	o	*d	dead	"
0.20	*	o	dead	"	"
0.22	*	o	"	"	"
0.40	*	****	"	"	"
Check	o	o	o	o	o
Check	o	o	o	o	o
Check	o	o	o	o	o

Legend: d = one leaf dead or dying; ** = permanently wilted; * = slightly wilted; o = unwilted; **** = very severely wilted; *** = severely wilted

shows the results with bean seedlings transferred at the stage when two simple leaves were expanded. It is evident from the data that a concentration of sodium chloride between 0.07 M. and 0.08 M. representing 2.94–3.35 atm. pressure is barely strong enough to reduce the absorption sufficiently to cause wilting. A solution of 3.76–4.15 atm. (0.09 M.–0.10 M.) seems to just balance the effective osmotic pressure developed in the roots of the plant. The time required to produce wilting varied with the concentration of the salt solution and the evaporating power of the air. An hour's ex-

posure was sufficient to cause wilting in certain cases. Longer exposures did not usually bring about sufficient readjustment in the plant to enable it to regain turgidity after it had once wilted. The stronger solutions did not always produce wilting, although the leaves of plants in these solutions acquired a peculiar color. Such solutions may have been toxic enough to injure the limiting membranes of the absorbing cells, enabling the plant to absorb the solution of sodium chloride.

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Summary

1. The stage of wilting known as permanent wilting is not necessarily a criterion of the critical point in soil moisture usually designated as the wilting coefficient.

2. Determinations of the distribution of moisture in the different regions of the bean seedling show that a fairly large proportion of the water in the plant is to be found in the lower part of the hypocotyl. In wilting, the transpiring leaves draw upon the water in the stem as soon as the supply from the roots is cut off.

3. Plants apparently permanently wilted, which had been cut at the soil surface, revived as many as 3 or 4 times when tested in the moist chamber. The results indicate that in the stages of wilting just preceding permanent wilting the plants are using stored water from their own tissues more than they are from the soil. Therefore the critical point for soil moisture may have been reached some time previous to permanent wilting.

4. Moisture determinations on numerous bean seedlings at permanent wilting indicated that the water content at that stage in wilting is lower for plants grown at low soil moistures than for those grown in soils with higher moisture contents.

5. The mechanical tissue per unit of leaf area was found to be greater in plants grown in soil at lower moisture contents. Thus leaves from such plants have greater mechanical rigidity, and a lowered water content is not indicated so quickly by the wilting of the leaf blade.

6. Water culture plants with roots adapted to tap water were wilted by transferring them to tap water to which sodium chloride had been added to bring the concentration to an 0.08 M. solution of NaCl (3.35 atm.). A 0.07 M. solution of NaCl (2.94 atm.) very rarely produced wilting.

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METHODS OF DETERMINING GLUCOSE AND FRUCTOSE IN CORN TISSUES[1]

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(WITH TWO FIGURES)

Introduction

In a series of studies on the physiology of corn, considerable attention has been given to the concentration of sugars in the different tissues of the plant, especially in relation to seasonal development. In these studies the total sugar content was differentiated into free reducing sugars and sucrose. It seemed desirable to carry the investigations a step further and determine the proportions of glucose and fructose which make up the free reducing sugars. Due to the similarity in chemical properties of these two sugars, however, procedures have not been standardized for the determination of either one of them in the presence of the other and sucrose in plant tissues. It consequently was necessary to make a preliminary survey of the available methods and to work out procedures which might be satisfactory for use with samples of corn tissues.

Three of the general methods which have been used for determining glucose and fructose in mixtures of glucose, fructose, and sucrose are: (1) the oxidation of glucose in alkaline solution with iodine, (2) the oxidation of fructose with Nyns' solution, a cupro-potassium carbonate solution, and (3) the method of analyzing a mixture of three sugars by determining the combined reducing power, polarization, and the direct determination of sucrose.

Materials and methods

In our studies of corn the tissue to be sampled was prepared by passing it through a food grinder, and it was desired to develop methods which might be applicable to two kinds of sampling. In one, the "Expressed sap method," the sap was expressed from 100 gm. of the ground tissue in a laboratory hydraulic press at a pressure of 5000 lb. per sq. in. Fifty-ml. aliquots of the sap were preserved by boiling with about 150 ml. of 95 per cent. alcohol and then making up to 200 ml. when cooled to room temperature. From determinations of the weight, total solids content, and specific gravity of the sap and the total moisture content of the tissue, the quantities of sugars found in the sap were calculated to percentages of fresh tissue.

[1] Based on investigations co-operative between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

The second kind of sample was obtained by the standard method of extracting 50 gm. of the ground tissue with 80 per cent. alcohol in a Soxhlet extractor. As the first method is quicker and simpler, the primary purpose of the extraction method was to check the accuracy of the expressed sap method.

Iodometric determination of glucose

An excellent review of the literature and a critical study of the factors involved in the oxidation of aldose sugars, such as glucose, by iodine in alkaline solution has been reported recently by Kline and Acree (**4**). The reaction is usually conceived of as occurring as follows:

$$RCHO + 3NaOH + I_2 \rightarrow RCOONa + 2NaI + 2H_2O$$

Determinations of glucose were made on portions of the same cleared aliquots of the alcoholic solutions as were used for determining the reducing power by means of Fehling's solution. In the clearing, 25 ml. of the alcohol-preserved sap sample, or 50 ml. of the alcoholic extract, were pipetted into a 100-ml. volumetric flask and the alcohol driven off on a steam plate. The syrupy residue was taken up in about 50 ml. of distilled water. To this, 0.5 ml. of a saturated solution of neutral lead acetate was added. The contents of the flask were made up to volume, shaken, and filtered through a folded filter. Sufficient potassium oxalate was added to precipitate the excess lead and the solution again filtered. It was found that any of the solutions which retained some color after undergoing this clearing process contained material which was oxidized by iodine, thus introducing considerable error. Such material, however, was found to be removed by treating the solution with about 1 gm. of decolorizing carbon (Baker and Adamson, Code BA 1551 bought from General Chemical Company) and filtering again. The effect of decolorizing with carbon on the indicated glucose contents of two solutions representing leaf tissues is shown in table I. That this effect of the carbon is not due to absorption of glucose is shown by the results with a pure glucose solution given in the same table.

The oxidation of the glucose was carried out as follows: a 25-ml. aliquot of the cleared and decolorized sugar solution was pipetted into a 250-ml. iodine flask and from 5 to 10 ml. of an approximately N/10 iodine solution run in from a burette. The quantity of iodine used depended upon the quantity of glucose present, since too great an excess causes over-oxidation. Five ml. were used for samples containing up to about 40 mg. of glucose (all samples of leaf tissue), and 10 ml. for those containing more (stem, tassel, and ear samples). A 10 per cent. sodium carbonate solution was added at the rate of 1.5 ml. for each 5 ml. of iodine and the flask placed in the dark for 15 minutes. Observations indicated that the oxidation was

TABLE I

EFFECT OF DECOLORIZING CLEARED SOLUTIONS ON THE QUANTITY OF IODINE USED

SAMPLE	TREATMENT	N/10 I USED	GLUCOSE
		ml.	*per cent.*
1. Leaf tissue	Without carbon	2.90	1.97
	Carbon added	2.10	1.43
2. Leaf tissue	Without carbon	3.00	2.04
	Carbon added	2.10	1.43
3. Glucose solution	Without carbon	2.55	1.73
	Carbon added	2.55	1.73

probably complete in 5 minutes, but, since there was no evidence of increased oxidation at the end of 15 minutes, that length of time was used as being more convenient. About 2 ml. of a freshly prepared starch solution was added and the excess iodine titrated with N/20 sodium thiosulphate. About 0.5 ml. of dilute sulphuric acid was added and the iodine thus released from combination as hypoiodite or iodate was titrated with the thiosulphate. Alternate additions of the dilute sulphuric acid and the N/20 sodium thiosulphate were made until no more iodine appeared on the addition of acid. This alternate addition of small quantities of acid and thiosulphate is probably much to be preferred to the addition of a rather large excess of acid at the start of the titration in working with solutions which contain sucrose. Otherwise, hydrolysis of the sucrose by the acid might provide glucose which would be immediately oxidized by the iodine present.

The weight of glucose oxidized by the iodine is calculated on the basis that 1 ml. N/20 iodine solution = 0.0045 gm. glucose.

DETERMINATION OF FRUCTOSE BY NYNS' METHOD

The determination of fructose by the use of Nyns' solution was carried out according to the procedure described by ZERBAN and SATTLER (**8**).

Determinations were made on aliquots of the same cleared and decolorized solutions used for the determination of glucose. It was found that the decolorizing step, however, was not necessary since the same results were obtained with or without the use of carbon.

To 10 ml. of the cleared solution in a 100-ml. centrifuge tube was added 10 ml. of distilled water and 50 ml. of Nyns' solution. The mixture was kept in a constant temperature water bath at 48.5–49.0° C. for 2.5 hours. The tube was then removed and centrifuged for 5 minutes. The supernatant liquid was carefully poured off, about 15 ml. of distilled water added from a wash bottle, and the tube again centrifuged. The supernatant

liquid was poured off and the copper oxide determined by dissolving in Bertrand's solution (20 per cent. sulphuric solution saturated with ferric sulphate) and titrating with N/20 potassium permanganate.

Although NYNS (5) claimed that when the factors of time, temperature, and concentration were closely guarded, the reagent showed a selective reactivity towards fructose in the presence of glucose and sucrose, this has not been substantiated by later workers, JACKSON (3), SCHUETTE and TERRILL (6), and ZERBAN and SATTLER (8). The last workers found, however, that with quantities of fructose ranging from 12 to 30 mg., and with about an equal quantity of glucose, approximately 13 mg. of glucose were required to reduce a quantity of copper equal to that reduced by 1 mg. of fructose. Preliminary determinations showed that the percentage of glucose in the free reducing sugars of corn tissues ranged from 50 to 70 per cent. The fructose-copper equivalents used in this study accordingly were determined on solutions containing 60 per cent. glucose and 40 per cent. fructose. The fructose-copper values are given in table II.

TABLE II

FRUCTOSE-COPPER EQUIVALENTS OF SOLUTIONS CONTAINING, IN ADDITION TO THE QUANTITIES OF FRUCTOSE STATED, 1.5 TIMES AS MUCH GLUCOSE

FRUCTOSE	CU
mg.	*mg.*
5	13.6
10	32.5
15	51.1
20	70.5
25	88.0
30	107.5
40	143.5

It was recognized that for proportions other than those mentioned above the quantity of copper reduced by 13 mg. of glucose might vary slightly from that reduced by 1 mg. of fructose. The sugar solutions from the samples of corn tissues, however, contained only from 2 to 18 mg. of fructose in the 10-ml. aliquots used for the fructose determination; hence, it was assumed that in solutions with such a limited range in concentration the error introduced by variations in this factor would be overshadowed by other errors.

For convenience in calculating results the chart shown as figure 1 was prepared. In this chart the abscissae represent the percentages of free reducing sugars, and the ordinates represent the milligrams of copper

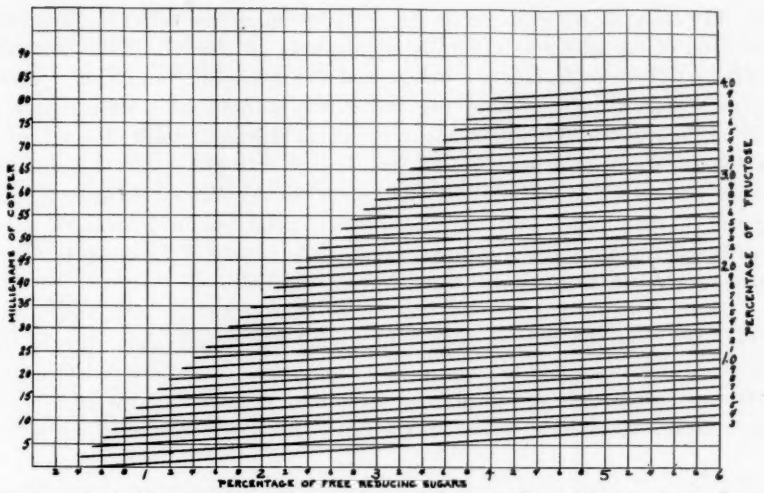

FIG. 1. Chart for calculation of percentage of fructose from determinations of percentage of free reducing sugars and milligrams of copper reduced from Nyns' solution.

reduced from Nyns' solution. By projecting the point of intersection of ordinate and abscissa for a given sample to the right hand margin, in a direction parallel to the sloping lines, the percentage of fructose may be read directly. This particular chart, of course, holds good only within the limits of the exact conditions used in this study with respect to dilution factors, etc.

POLARIZATION METHOD

A method of calculating the percentages of glucose, fructose, and sucrose in a mixture of the three sugars from determinations of reducing power, polarization, and the direct determination of sucrose has been described by BROWNE (1).

As modified for use with samples of corn tissues the calculation was based upon the following determinations: (1) the quantity of free reducing sugars by reduction of Fehling's solution; (2) the quantity of sucrose as determined by the additional reducing power after the inversion of sucrose with invertase; and (3) the polarization of the sugar solution.

The free reducing power was calculated as invert sugar and no further correction was made for the difference between the reducing ratios of glucose and fructose, since the concentrations of reducing sugars were relatively low and the range in proportions of glucose and fructose rather narrow (50 to 70 per cent. glucose).

The samples were prepared for polarization in the following manner: 100 ml. of the alcoholic expressed sap sample or 250 ml. of the alcoholic extract were transferred to a beaker and the alcohol driven off on a steam plate. The syrupy residue was taken into solution and washed into a 25-ml. volumetric flask with about 20 ml. of distilled water; 0.5 ml. of a saturated solution of neutral lead acetate then was added and the contents made up to volume and shaken. The solution was filtered through a good grade of filter-paper and the clear filtrate polarized in a 200-mm. tube, using a Schmidt-Haensch saccharimeter. The source of light was a microscope lamp containing a 400-watt stereopticon bulb.

The calculated polarization due to the sucrose present was subtracted from the observed polarization, thus giving the polarization due to the mixture of glucose and fructose. The proportions of glucose and fructose, expressed as percentage of glucose, were then obtained by use of a chart such as is illustrated in figure 2. On this chart is represented the rotation

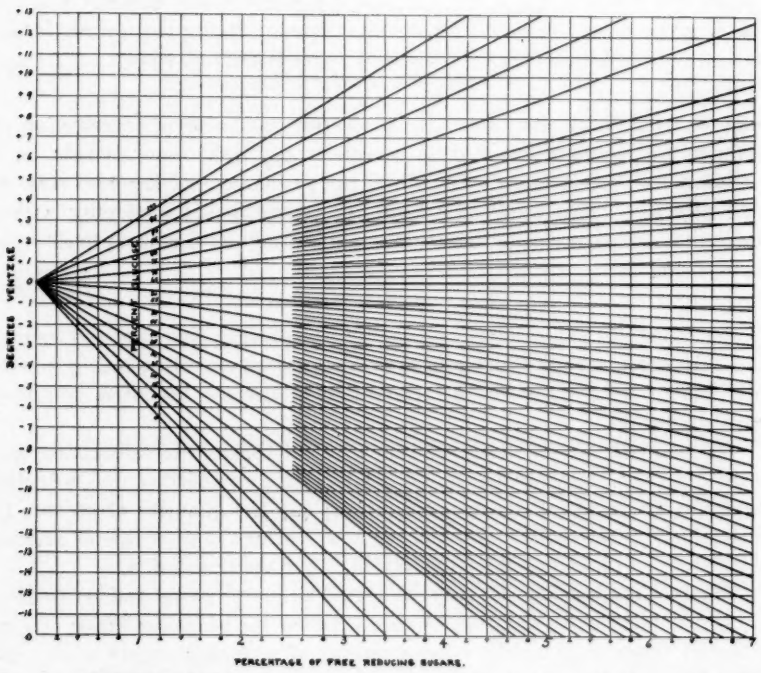

FIG. 2. Chart for calculation of percentage of glucose in free reducing sugars from determinations of free reducing sugars and their rotation.

in degrees Ventzke of all possible proportions of glucose and fructose in sugar solutions containing up to 7 per cent. free reducing sugars, calculated from their specific polarizations. On this chart the ordinates represent the rotation in degrees Ventzke due to the free reducing sugars and the abscissae represent the percentages of free reducing sugars. At the point of intersection of ordinate and abscissa for a given sample the proportion of glucose and fructose was read, expressed as percentage of glucose in the free reducing sugars.

EXAMPLE.—A sample containing 2.38 per cent. free reducing sugars and 6.02 per cent. sucrose was found to have a rotation of + 22.8° V. Since the rotation of a 6.02 per cent. sucrose solution is + 23.1° V., the 2.38 per cent. of free reducing sugars has a rotation of − 0.3° V. As shown by the chart, figure 2, 62.2 per cent. of the free reducing sugars was glucose, and therefore the sample contained 1.48 per cent. glucose and 0.90 per cent. fructose.

ANALYSIS OF PURE SUGAR SOLUTIONS

As a check on the accuracy of the three methods of determining glucose and fructose a series of 10 samples containing known quantities of glucose, fructose, and sucrose was analyzed by each of the methods. The concentrations of sugars in these samples were representative of those actually found in samples of corn tissues. The sugars were Pfanstiehl brand prepared by the Special Chemicals Co.

The samples were prepared in such a way as to simulate the preparation of the expressed sap samples previously described. The quantities of sugars for each sample were weighed out, placed in 200-ml. graduated prescription bottles, and 50 ml. of distilled water added to dissolve the sugars. The bottles were then filled nearly to the 200-ml. mark with 95 per cent. alcohol and heated in a water-bath for 30 minutes. After cooling to room temperature they were filled to the 200-ml. mark, thoroughly shaken, and set aside for analysis. In analyzing these solutions exactly the same procedures were followed as with the expressed sap samples, including the addition of neutral lead acetate and decolorizing carbon.

The composition of the samples as prepared and found by analysis is reported in table III. The determinations made were: reducing sugars before and after inversion of sucrose with invertase as measured by reduction of Fehling's solution; glucose by the iodine method; fructose by Nyns' solution; and the proportion of glucose and fructose by the polarization method, the results of the last determination being reported as glucose.

The mean quantity of glucose present in the samples was 1.71 per cent. while that indicated by the iodine method was 1.735 and by the polarimeter method was 1.728. The average quantity of fructose indicated was 1.15

TABLE III

COMPOSITION (GRAMS PER 100 ml.) OF PREPARED SOLUTIONS OF PURE SUGARS

SAMPLE	AS PREPARED	COMPOSITION OF SAMPLES AS FOUND BY THE METHOD OF ANALYSIS INDICATED				
		GLUCOSE		FRUCTOSE	FEHLING'S SOLUTION	
		IODINE	POLARIZATION	NYNS'	FREE REDUCING SUGARS	SUCROSE
no.		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	Glucose 0.60 Fructose 0.40 Sucrose 0.50	0.57	0.60	0.42	1.01	0.54
2	Glucose 1.20 Fructose 0.80 Sucrose 0.50	1.20	1.20	0.78	1.97	0.51
3	Glucose 1.20 Fructose 0.80 Sucrose 1.50	1.19	1.17	0.80	1.97	1.58
4	Glucose 1.50 Fructose 1.00 Sucrose 0.50	1.58	1.52	0.96	2.45	0.54
5	Glucose 1.50 Fructose 1.00 Sucrose 1.00	1.55	1.49	0.98	2.47	1.05
6	Glucose 1.50 Fructose 1.00 Sucrose 2.00	1.54	1.49	1.01	2.47	2.03
7	Glucose 1.80 Fructose 1.20 Sucrose 1.00	1.88	1.80	1.23	2.95	1.08
8	Glucose 2.40 Fructose 1.60 Sucrose 6.50	2.38	2.55	1.66	4.06	6.34
9	Glucose 2.70 Fructose 1.80 Sucrose 2.50	2.70	2.70	1.78	4.39	2.49
10	Glucose 2.70 Fructose 1.80 Sucrose 5.00	2.76	2.76	1.84	4.47	4.97
	Mean Glucose 1.71 Fructose 1.14	1.735	1.728	1.15		

per cent. whereas the samples actually contained 1.14 per cent. This close agreement, both as to means and individual samples obtained under the conditions of the experiment, indicated that all three methods might be expected to give results well within the limits of accuracy desirable with samples of corn tissues.

Comparison of methods with corn tissues

The three methods were compared on corn tissues during the season of 1930. The variety of corn used was Burr-Leaming, a double cross. For convenience in sampling, the plant was divided into leaves and stem, and when the plants attained a height of approximately 120 cm. the stem was divided into upper, middle, and lower parts.

The sugar content of the samples ranged from leaf tissues containing 0.9 per cent. to stem tissues containing 5.5 per cent.

Expressed sap and alcoholic extraction samples as previously described were used. The results obtained by each of the methods were expressed as percentages of glucose on a fresh weight basis. In the case of Nyns' method the data were calculated to this basis by subtracting the quantity of fructose found from the free reducing sugars, the difference being regarded as glucose.

Single determinations were made on each of the duplicate samples taken from each lot of corn tissue. This naturally tended to make the differences between the comparable values relatively large since they reflect not only the variation due to analytic technic but also that due to sampling. The dependability of the averages representing the corn was increased by this procedure.

Results

The three methods were compared with regard to: (1) agreement in determining the mean glucose content of all samples; (2) agreement in determining the glucose content of different kinds of tissue in varying stages of development; and (3) the relative precision of the different methods in determining the glucose in duplicate samples.

Expressed sap samples

The percentages of glucose in samples of expressed sap as determined by the different methods are shown in table IV. The mean of the 30 samples was 1.767 per cent. for the iodine method, 1.780 per cent. for Nyns' method, and 1.919 per cent. for the polarization method. The significance of the differences among these means was tested, using Fisher's (**2**) formula, $t = \frac{\bar{x} - \bar{x}_1}{s}\sqrt{n}$, and the probability tables given by Student (**7**). Although the difference between means based on the iodine and Nyns' methods was only 0.013 per cent. and well might be due to chance, the odds

TABLE IV

PERCENTAGES OF GLUCOSE IN TWO KINDS OF SAMPLES OF CORN TISSUES AS DETERMINED BY THREE METHODS

DATE OF SAMPLING	TISSUE	EXPRESSED SAP SAMPLES METHOD						ALCOHOLIC EXTRACTION SAMPLES METHOD					
		IODINE		NYNS'		POLARIZATION		IODINE		NYNS'		POLARIZATION	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
July 7	Leaves	0.69	0.62	0.54	0.52	0.61	0.59	0.73	0.73	0.49	0.48	0.64	0.59
	Stems	1.26	1.23	1.24	1.17	1.21	1.19	.....	.....	.....	.....	.....	.....
14	Leaves	0.91	0.86	0.69	0.68	0.71	0.71	.....	.....	.....	.....	.....	.....
	Stems	1.71	1.62	1.71	1.62	1.81	1.77	.....	.....	.....	.....	.....	.....
22	Leaves	0.98	0.89	0.93	0.86	1.24	1.13	0.76	0.79	0.87	0.85	1.10	1.14
	Stems	2.01	1.94	2.16	1.87	2.43	2.32	1.90	2.10	2.03	1.99	2.30	2.32
29	Leaves	0.91	0.86	1.01	0.98	0.99	0.98	.....	.....	.....	.....	.....	.....
	Upper stems	2.79	2.63	3.01	2.78	2.86	2.81	.....	.....	.....	.....	.....	.....
	Lower stems	2.40	2.36	2.65	2.62	2.69	2.58	.....	.....	.....	.....	.....	.....
Aug. 5	Leaves	1.12	1.12	1.15	1.08	1.21	1.15	1.24	1.20	1.00	1.02	1.06	1.11
	Upper stems	2.70	2.67	2.82	2.72	3.00	2.89	.....	.....	.....	.....	.....	.....
	Middle stems	2.33	2.27	2.42	2.40	2.69	2.61	.....	.....	.....	.....	.....	.....
	Lower stems	2.38	2.35	2.45	2.33	2.63	2.53	2.19	2.19	2.46	2.48	2.35	2.40
11	Leaves	1.15	1.13	1.14	1.09	1.21	1.12	1.13	1.12	1.18	1.16	1.16	1.23
	Sheaths	2.28	2.28	2.30	2.18	2.41	2.39	.....	.....	.....	.....	.....	.....
	Upper stems	2.59	2.54	2.78	2.77	2.99	2.93	.....	.....	.....	.....	.....	.....
	Middle stems	2.54	2.49	2.67	2.65	2.82	2.81	.....	.....	.....	.....	.....	.....
	Lower stems	2.46	2.27	2.41	2.33	2.58	2.56	2.30	2.30	2.50	2.64	2.55	2.79
18	Leaves	1.64	1.38	1.38	1.36	1.73	1.58	1.53	1.63	1.30	1.46	1.29	1.40
	Upper stems	2.39	2.29	2.29	2.24	2.52	2.45	.....	.....	.....	.....	.....	.....
	Middle stems	2.22	2.17	2.13	2.07	2.33	2.28	.....	.....	.....	.....	.....	.....
	Lower stems	2.29	2.24	2.23	2.08	2.29	2.17	2.31	2.31	2.30	2.30	2.35	2.31
25	Leaves	1.01	0.97	0.94	0.87	0.91	0.87	0.89	0.88	0.90	0.86	0.85	0.83
	Upper stems	2.21	2.18	1.88	1.85	2.10	2.07	.....	.....	.....	.....	.....	.....
	Middle stems	1.85	1.82	2.06	2.03	2.28	2.28	.....	.....	.....	.....	.....	.....
	Lower stems	2.12	2.03	2.23	2.17	2.36	2.34	2.00	2.00	2.40	2.28	2.49	2.56
Sept. 8	Leaves	1.05	0.97	1.15	1.06	1.26	1.17	0.96	1.14	1.00	1.00	1.16	1.18
	Upper stems	1.27	1.26	1.43	1.41	1.64	1.60	.....	.....	.....	.....	.....	.....
	Middle stems	1.28	1.24	1.34	1.21	1.44	1.32	.....	.....	.....	.....	.....	.....
	Lower stems	1.39	1.38	1.35	1.30	1.48	1.48	1.45	1.38	1.48	1.46	1.46	1.48
	Mean	1.767		1.780		1.919		1.506		1.534		1.619	

being 3:1, the odds were infinite against random differences as large as those between the means determined by the polarization method on the one hand and those determined by the iodine method and Nyns' method on the other.

The product moment coefficients of correlation for glucose content in the different samples were: for the iodine and Nyns' methods, 0.984 ± 0.004; for the iodine and polarization methods, 0.980 ± 0.005; and for the Nyns' and polarization methods, 0.991 ± 0.002.

The standard error of a single result, $\sqrt{\frac{\Sigma d^2}{(m-1)(n-1)}}$ is used to measure the precision of the different methods. In this formula Σd^2 indicates the sum of the squared deviations of the duplicates from their mean for each of the n $(=30)$ samples and m is the number in the group, here the two of each pair. The standard errors were 0.060, 0.069, and 0.053 for the iodine, Nyns', and polarization methods, respectively.

It may be concluded from this comparison of the three methods as applied to expressed sap samples that the correlation is high enough to show conclusively that the methods could be used interchangeably for determining variations due to kind and stage of development of tissues; furthermore, the precision of each of the methods was satisfactory inasmuch as all the errors are less than four per cent. of the total glucose content. On the other hand, the results obtained by the polarization method were significantly higher than those by the iodine and Nyns' method, the latter two being in good agreement.

Alcoholic extraction samples

The applicability of the three methods to alcoholic extraction samples was determined on 13 samples of the series. The percentages of glucose by the different methods are included in table IV.

The means for the 13 samples were: 1.506 per cent. for the iodine method, 1.534 per cent. for Nyns' method, and 1.619 per cent. for the polarization method. The odds were 20:1 and 90:1 against random differences as large as those between the means for the polarization method on the one hand and the iodine method and Nyns' method on the other. The difference between the means for the iodine and Nyns' methods was 0.028 with odds of only 2:1 against random differences this large. In the alcoholic extraction samples the product moment coefficients of correlation for the Nyns' method with the iodine and the polarization methods were both 0.986 ± 0.005. That for determinations by the polarization and iodine methods was 0.959 ± 0.015. The standard errors were 0.061, 0.051, and 0.062 for the iodine, Nyns' and polarization methods, respectively.

The same general conclusions may be drawn from this comparison of the three methods applied to alcoholic extraction samples as were arrived

at with expressed sap samples; namely, that while the methods can be used interchangeably for determining variations due to kind and stage of development of corn tissues and also are satisfactory from the point of view of precision, the mean of the results obtained by the polarization method is significantly higher than those based on the iodine and Nyns' methods, the latter two being in good agreement.

Comparison of analyses of expressed sap and alcoholic extraction samples

With both kinds of samples the polarization method gave a mean glucose content which was significantly higher than those by the iodine and Nyns' methods; all three methods agreed reasonably with respect to the variation in samples due to differences in kind of tissue or seasonal development and also with respect to the precision in determining the glucose content in duplicate samples. Since the alcoholic extraction samples were primarily to check the accuracy of the results obtained by analyzing the expressed sap and then calculating the glucose content of the original tissue, it seemed desirable to make a direct comparison of the analyses of the 13 comparable lots of tissue for which data on both kinds of samples were available.

There were no important differences between the means for any method in the two kinds of samples. Such differences as there were well might have been due to chance, the odds not exceeding 4 : 1 in any comparison.

The product moment coefficients of correlation for glucose content in the two kinds of samples ranged from 0.984 to 0.996. These high correlations in connection with the close agreement of mean values are sufficient evidence that expressed sap samples may be used in place of alcoholic extraction samples without error.

Determination of free reducing sugars

The determinations of glucose by oxidation with iodine and of fructose with Nyns' solution appear to give reliable results. These methods then might well replace the usual determination of free reducing sugars with Fehling's solution in some investigations.

To test this possibility, the determinations of free reducing sugars with Fehling's solution in the series of 30 expressed sap samples were compared with the sum of glucose by the iodine method and fructose by Nyns' method. The data are given in table V.

The mean of the 30 samples was 3.140 per cent. as determined with Fehling's solution and 3.127 per cent. for the sum of glucose and fructose. The difference is only 0.013, and no larger than might be expected from random sampling in one out of every 4 trials.

TABLE V

FREE REDUCING SUGAR CONTENT OF EXPRESSED SAP FROM CORN TISSUE SAMPLES AS DETERMINED BY TWO METHODS

DATE OF SAMPLING	TISSUE	FEHLING'S SOLUTION	GLUCOSE, IODINE + FRUCTOSE, NYNS
		per cent.	*per cent.*
July 7	Leaves	0.920	1.045
	Stems	2.170	2.210
14	Leaves	1.135	1.335
	Stems	3.380	3.380
22	Leaves	1.525	1.565
	Stems	3.875	3.835
29	Leaves	1.560	1.450
	Upper stems	5.300	5.115
	Lower stems	4.735	4.480
Aug. 5	Leaves	1.955	1.960
	Upper stems	5.575	5.490
	Middle stems	4.590	4.480
	Lower stems	4.310	4.285
11	Leaves	1.865	1.890
	Sheaths	4.025	4.065
	Upper stems	5.220	5.010
	Middle stems	4.650	4.505
	Lower stems	3.880	3.875
18	Leaves	2.170	2.310
	Upper stems	4.125	4.200
	Middle stems	3.615	3.710
	Lower stems	3.500	3.610
25	Leaves	1.375	1.460
	Upper stems	3.255	3.585
	Middle stems	3.385	3.175
	Lower stems	3.610	3.485
Sept. 8	Leaves	1.880	1.785
	Upper stems	2.390	2.235
	Middle stems	2.030	2.015
	Lower stems	2.190	2.250
	Mean	3.140	3.127

There is a high correlation for the free reducing sugar contents as determined by the two methods ($r = 0.997$). These methods of determining total free reducing sugars accordingly are in excellent agreement in all ways.

Discussion

In the comparison of the three methods of determining glucose in the expressed sap and alcoholic extraction samples of corn tissues used in this

study it was shown that the polarization method gave results which were significantly higher than those obtained by either the iodine or Nyns' method. All attempts to discover the reason for this discrepancy have failed. The most likely source of error first appeared to be that occasioned by the defecation of materials with neutral lead acetate in the 25-ml. volumetric flask. The sugar concentration of the solution presumably is increased in direct proportion to the volume occupied by this precipitate. Moreover, as would be expected, the tendency of the polarization method to give higher results was not observed in measuring the recovery of pure sugars from solutions of known concentration. On the other hand, it would be expected that this tendency toward higher values would be most marked with those solutions which contained the largest quantities of precipitable material; namely, those representing leaf tissues. The data in table I, however, do not seem to substantiate this idea. Instead, those solutions having the higher percentages of sugars, the stem samples, are the ones which show the greatest discrepancies.

Polarization has the advantage of simplicity in technique and may well serve in preliminary studies. Moreover, the error obtained in the use of this method is not large, the difference between the means by polarization and the iodine methods being 0.142 per cent. and that between polarization and Nyns' 0.139 per cent. on a fresh weight basis, differences of less than 8 per cent. of the total glucose. The largest individual difference, that of 0.445 per cent., was found between the polarization and iodine methods on August 25 in middle stem tissue, containing about 2 per cent. of glucose.

The chief disadvantages of polarization are: (1) the large quantity of extract necessary to get a sufficient concentration of sugar for a saccharimeter reading; and (2) some extracts, particularly of leaf tissues, are so highly colored after clearing that it is extremely difficult to obtain readings with the saccharimeter even with a powerful source of light.

The oxidation of glucose by iodine is undoubtedly the most sensitive and rapid method of the three compared. Since iodine is readily absorbed or reduced by many materials contained in plant extracts, however, unusual care must be taken in the clearing and decolorizing processes. Also, the quantity of iodine solution used must be carefully regulated since too great an excess causes over-oxidation and with an insufficient excess there is under-oxidation.

The method of Nyns is much less sensitive than the iodine method and the conditions under which the oxidation is carried out fit the method well to routine analysis. Furthermore, the apparatus and most of the reagents required are those also used in the determination of reducing power by Fehling's solution. The most serious objection to the method is that the reagent is not selective for fructose, but also oxidizes glucose, making the

calculation quite complicated. It would seem, however, that this method will be most valuable when used with samples, such as apple tissue in which fructose is the predominating free reducing sugar. Moreover, the results obtained in this study indicate that with the relatively small quantities of sugar found in most plant tissues, and with a rather constant glucose-fructose ratio, the calculation need not be so complicated in arriving at analyses which are within the range of experimental error of such materials.

Summary

1. With pure sugar samples all three methods gave results which were well within the limit of accuracy desired for use with samples of corn tissues.

2. The iodine and Nyns' methods gave values for glucose which were in excellent agreement in every way when used with expressed sap and alcoholic extraction samples.

3. The results obtained by the polarization method with the corn samples were consistently higher, the mean values being about 8 per cent. higher. The precision of the method, however, was equal to the other two and probably has some value in preliminary studies.

4. Either expressed sap or alcoholic extraction samples could be used equally well with all three methods.

5. Free reducing sugars may be determined from the sum of glucose by iodine and fructose by Nyns' methods with the same degree of accuracy as directly by the reducing power with Fehling's solution.

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INVESTIGATIONS OF THE HARDINESS OF PLANTS BY MEASUREMENT OF ELECTRICAL CONDUCTIVITY[1]

S. T. DEXTER, W. E. TOTTINGHAM, AND L. F. GRABER

In a preliminary report, DEXTER, TOTTINGHAM and GRABER (3) have described a method of estimating the hardiness of plants as applied to three varieties of alfalfa, in which exosmosis of electrolytes from suitably frozen tissue into distilled water is determined electrometrically, and the magnitude of the electrical conductivity associated with the known hardiness of the samples. They found a progressive hardening of Grimm alfalfa, a hardy variety, as the winter season approached, and little or no hardening in a very tender strain, Hairy Peruvian. Utah Common alfalfa was intermediate in response.

The principle of increased permeability and electrical conductivity of injured tissue is a familiar one in plant physiology, while the method of electrical conductance as a measure of changing electrolyte concentration has been more or less standard for many years. (See OSTERHOUT (16); DIXON and ATKINS (4); HOAGLAND and DAVIS (9); HIBBARD and MILLER (6); HOTTES and HUELSEN (10); PANTANELLI (17); MERRILL (13); STILES and JØRGENSON (21).) In the papers listed above the principle has been applied to injuries resulting from freezing, from the action of various chemicals, from pathological causes, and from mechanical rupture.

In view of the fact that the chemical and physical theories of winter-hardiness of plants have been amply discussed in recent publications, and since the bibliography has been so fully covered there, the consideration of this phase of the problem will be dismissed by reference to several papers which furnish suggestive discussions and inclusive citations of the literature. MAXIMOW (12) has reviewed the various physical and chemical studies of the hardiness problem in which compositional or osmotic and colloidal properties of plants are shown to be associated with hardiness. He cites, as well, numerous recent investigations in which growth recovery from controlled freezing treatment is used to estimate the degree of hardiness. NEWTON (15), WEIMER (22), STEINMETZ (20), ROSA (18) and CHANDLER (2) present excellent discussions, full bibliographies and additional data in regard to several chemical and physical methods of investigating the hardiness of plants. It may be stated briefly that most, if not all, of the theories of winter-hardiness are built around the idea of the water-relations of the plant, and that structural, osmotic or colloidal pro-

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tection from water-withdrawal, or ice-formation form the basis of explanation for individual or varietal differences in hardiness.

The positive recognition of hardiness in plants, and the practical estimation of the degree of injury from freezing are matters which do not seem to have been adequately considered, nor have the various studies furnished convenient means for their accomplishment. MAXIMOW (**12**) in concluding his review of the various methods previously put forth states "we may observe that, notwithstanding a series of successful attainments, the study of the internal factors conditioning hardiness has not yet established any reliable and easily determinable symptoms for use in breeding work. For this reason, refrigerating machines—are being more and more used for the acceleration of the breeding of frost resistant varieties." Thus, the recent work of STEINMETZ (**20**), WEIMER (**22**), MARTIN (**11**), STEINBAUER (**19**), BRIERLEY and HILDRETH (**1**), HILL and SALMON (**8**), HARVEY (**5**) and others shows that varieties of alfalfa, wheat, red clover, apples and other plants can be distinguished from one another on a hardiness basis when suitably frozen after a period of hardening. In these experiments, the injury to the tissue exposed to cold temperatures is estimated by the degree of growth recovery or by microscopic observation.

The work of DEXTER, TOTTINGHAM and GRABER (**3**) has indicated that injury from controlled freezing treatments may be conveniently determined by measurement of the degree of exosmosis of electrolytes and that alfalfa varieties so examined fall into the proper order of hardiness. It is true that such measurements have not yet been adequately related to the rate or degree of recovery from freezing injury, but specific determination of injury in itself is an important step in a critical study of hardiness and its related problems.

The rapidity with which the estimation of injury can be accomplished and the convenience of the precise numerical values obtained by electrical conductance, makes the application of the method to plants other than alfalfa a matter of some interest. Moreover, as suggested above, a modification of the method might well be devised in which conductivity measurements of the tissues themselves would be made, subsequent to freezing injury, since increased conductivity might be anticipated in the injured tissues. To these ends, a series of experiments has been performed, investigating some important aspects of the hardiness problem as related to several crops under various environments.

Investigations

CONDITION OF THE ROOTS IN THE FIELD IN WINTER

In order to investigate the condition of alfalfa roots in the field in winter, samples of roots were dug from the deeply frozen ground on January

10, 1930. These roots were two years old, from plats which had received but two cuttings in the summer of 1929. Twenty-gram samples of roots were prepared in the usual manner (see Dexter, *et al.* (**3**) for details) and without further freezing treatment beyond that received in the field, were exposed to exosmosis into 50 cc. of water at 25° C. for ten hours. Table I gives the results of this trial.

TABLE I

SPECIFIC CONDUCTIVITY ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACT FROM ALFALFA ROOTS FROZEN ONLY IN THE FIELD. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

VARIETY	SPECIFIC CONDUCTIVITY
Grimm	360
Utah Common	634
Hairy Peruvian	1690

These data give definite evidence of severe field injury in the case of Hairy Peruvian, considerable injury in the case of Utah Common and but very little injury in the case of Grimm alfalfa. Conductivity of checks, in the fall, on unfrozen tissue approximated 200. Field counts in the following spring indicated almost complete loss of plants in the Hairy Peruvian plats, about 50 per cent. loss in the Utah Common plats, and under 10 per cent. loss in the Grimm plats.

A similar experiment on the same date with three varieties of winter wheat indicated precisely the same condition. The magnitudes of the electrical conductivity values and hardiness were correlated with larger exosmosis from the tenderer strains. From a ten-gram sample of crowns, exosmosis for seven hours into 40 cc. of water gave specific electrical conductivities ($\times 10^6$) of 423 for Wisconsin Pedigree no. 2 wheat, 478 for Michigan Amber wheat and 558 for Dawson wheat. These values are quite in accord with the order of hardiness of these varieties as determined by field trials at the Wisconsin Experiment Station.

EXPERIMENTS ON GRAINS GROWN IN THE GREENHOUSE

To continue the work throughout the winter, several varieties of small grains were planted in beds and in wooden flats in the greenhouse on Jan. 27, 1930. The temperature of the greenhouse varied considerably and rarely reached a temperature as low as 60° F. during the first few weeks. Preliminary studies indicated very marked hardening effects in these greenhouse plants, with much lower exosmosis and electrical conductivity from frozen wheat crowns which had been previously exposed for a few hours to temperatures approximating 35° F.

Consequently a series of experiments was run to test several varieties in respect to hardening in a more extensive way. Samples were prepared, on each date, by removing the roots and a portion of the leaves from the individual plants leaving a clean, etiolated crown about an inch long. From such material, without washing, 2.5 gm. samples were secured, placed in Pyrex tubes and frozen in an air chamber at −9° C. for 1.5 hr. After thawing at room temperature, 20 cc. of distilled water were added to each tube and exosmosis permitted to proceed at 25° C. for 10 hr. before conductivity measurements were made. Samples were first taken on March 7 from the greenhouse maintained at approximately 60° F. and subjected to the freezing-exosmosis treatment described. The house was then cooled as much as the prevailing weather would permit. It rarely reached a temperature below 50° F. although on two successive days the house was cooled to below 40° F. for a few hours. On these two cooler days, flats containing lumps of soil in which the wheat was growing were placed outside for an additional hardening treatment at a temperature of about 32° F. Samples were prepared from the greenhouse material and from the outside plats on March 15. Table II presents the data for the three hardening treatments. The first treatment represents, roughly, wheat grown for six weeks in a 60° F. house; the second, wheat with an additional week in a 45° F. house; and the third treatment, wheat with a further additional hardening of 12 or 15 hr. at a temperature approximating 32° F.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF CROWNS OF SEVERAL VARIETIES OF WHEAT AND RYE FROZEN AT −9° C. FOR 1.5 HOURS. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

VARIETY	UNFROZEN TISSUE FROM SECOND HARDENING TREATMENT (MARCH 15)	FROZEN AFTER HARDENING TREATMENT		
		FIRST (60° F.) (MARCH 7)	SECOND (45° F.) (MARCH 15)	THIRD (32° F.) (MARCH 15)
Wheat				
Wisconsin Pedigree no. 2	141	1764	920	726
Ashkoff	164	1123	987	747
Gladden	154	1362	765	774
Honor	86	1630	765	695
Marquis	305	2355		
Progress	324	2415		
Rye				
Wisconsin Pedigree no. 2	168	882		
Spring rye	303	2155		

That hardening occurred in these winter wheats as the temperature was lowered is clearly shown. However, no definite correlation of freezing injury and electrical conductivity with varietal hardiness is evident at any stage of this hardening treatment. It is very apparent from the figures given that the hardening process was by no means complete. From data obtained at a later date from field plats (see table V) this hardening treatment would appear to be insufficient to develop the characteristic varietal differences in hardiness which occur in the field under normal fall and winter environment. It is interesting to observe that, although the hardy types, Wisconsin Pedigree no. 2 wheat and Ashkoff wheat, had assumed a very recumbent type of growth, they were in an unhardened condition on March 7 and were very sensitive to freezing. The very high values for conductivity which are found with the unfrozen tissues of spring grains are of interest and are due perhaps to the very succulent type of tissue produced under greenhouse conditions.

Chloride tests on the exudates from the wheat crowns gave values varying in a manner comparable to the conductivity values, and could be used to distinguish, at least roughly, the hardened from the unhardened tissues.

Further studies of the hardiness of alfalfa in the autumn of 1930

On June 27, 1930, several additional varieties of alfalfa were seeded in rows and in field plats for study the following season. In the continuation of the work with alfalfa, several modifications of the procedure seemed necessary and desirable. The June seedlings were of such a size that no attempt was made to have equal numbers of plants in the twenty-gram samples from the various varieties. From the row cultures it was impossible to obtain strictly comparable samples. Poor stands in some of the rows and excessive crowding in others gave roots and plants of widely divergent sizes and development. To the contrary, samples from the field plats were comparable, and gave satisfactory checks on duplicates. In the plats used in 1929 (now three years old) winter-killing and insect-injury of the roots made sampling somewhat difficult. No attempt was made to use eight roots in samples weighing twenty grams, as in 1929 (See Dexter *et al.* (**3**), but the samples consisted, in 1930, of twenty gm. of representative root tissue without reference to the number of roots.

Additional low temperature facilities in 1930 permitted the use of a freezing chamber (7 cu. ft.) stirred with an electric fan, and, on occasion, of a large liquid bath. Either of these could be held at the desired temperature by thermostatic controls. The tissues were permitted to thaw by immersion of the tubes in an ice-water bath. After an hour or two, water at 0° C. was added to the tubes and exosmosis was permitted to continue for a period of twenty hours at this temperature, when electrical conduc-

tivities were determined. Exosmosis is somewhat less complete in this method than in the method used in 1929 (10 hours at 25° C.) but there is obviously less opportunity for complicating fermentations. In addition, the values given, being specific conductivities at 0° C. rather than at 25° C., must be multiplied by a constant (about 1.82) if they are to be compared with the previous figures. However, among themselves they are entirely comparable.

A series of row cultures of ten hardy strains introduced by H. L. WESTOVER, of the U. S. Department of Agriculture, was examined throughout the season. These varieties might be classed as Turkestan since they were selected in various parts of that area. All of these varieties were of a more or less dormant type. Every variety showed the same hardening effect that was reported in 1929, although some reached much lower values than others. From original conductance of approximately 750 for all varieties on October 1, final values of from 286 to 466 were reached on December 5. The varieties were rather uniform in hardiness on December 5, except for two or three varieties from the Khiva district which were hardier than the rest, as anticipated by WESTOVER. Since the varieties are unnamed, no value can be gained in this paper by enumerating the many conductivities taken on the various dates throughout the fall.

Another series of hardy varieties, in field plats, were compared as shown in table III. Several tender varieties are included for comparison. The data in this table indicate wide differences in hardiness between the very

TABLE III

SPECIFIC CONDUCTIVITIES (x 10^6, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF ALFALFA ROOTS FROZEN 4 HOURS IN A LIQUID BATH AT −10° C. THE ROOTS WERE DUG ON DEC. 10, 1930, FROM SLIGHTLY FROZEN GROUND. AN INTERVAL OF 20 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	AGE OF PLANTS	SPECIFIC CONDUCTIVITY
Canadian Variegated	Seedling 5 mos.	377
Grimm	Seedling "	384
Ladak	Seedling "	322
Hardistan	Seedling "	281
Turkestan	Seedling "	364
Cossack	Seedling "	384
Hairy Peruvian (row)	Seedling "	593
Hairy Peruvian (plat)	Three years	513
Argentine (row)	Seedling 5 mos.	546

dormant types, as Hardistan or Ladak, and the vegetative types such as Hairy Peruvian and Argentine. Duplicate samples from the field plats

gave good check values for conductivity whereas the row cultures are sometimes uncertain in this respect.

Application of the test to red clover varieties

On December 9, red clover roots were dug from field plats which had been planted with barley as a nurse crop the preceding spring. The samples were prepared in the usual manner, using twenty-gram samples from the thoroughly washed and surface-dried roots. These samples were placed in Pyrex tubes, frozen four hours in a liquid bath at $-10°$ C. and thawed at 0° C. in ice-water. Exosmosis into 50 cc. of water at 0° C. was permitted to continue for 20 hours. Table IV indicates that a difference in hardiness existed between the Wisconsin strain grown in this state for fifty years, a variety from Missouri, and one imported from Italy. These results show that this test will differentiate the hardiness of roots of these three varieties of red clover.

TABLE IV

Specific conductivities ($\times 10^6$, 0° C.), expressed in reciprocal ohms, of water extracts of clover roots frozen 4 hours in a liquid bath at $-10°$ C. The roots were dug on Dec. 9, 1930, from slightly frozen ground. An interval of 20 hours was allowed for exosmosis at 0° C.

Variety	Specific conductivity
Wisconsin	730
Missouri	900
Italy	1171

Application of the test to winter-wheat sown in field plats at the usual rate per acre

Samples were prepared in the manner previously described, from plants dug from field plats of wheat on three dates in the fall. The samples consisted of 100 gm. of crowns. These were frozen on open screens in front of an electric fan in the freezing chamber. They were thawed in beakers immersed in ice-water. After thawing, the samples were placed in flasks, to which 750 cc. of water at 0° C. were added. Exosmosis was allowed to proceed for 16 hours at 0° C. Table V presents the results of these trials. In this table it should be noted that the freezing treatment was made more severe on December 6 than on the previous dates, since pronounced hardening had by this time rendered the plants of the hardier varieties unsusceptible to the milder freezing treatment. The table indicates the same tendency that has been described before. Following the hardening process

TABLE V

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF WINTER WHEAT CROWNS FROZEN AS INDICATED. AN INTERVAL OF 16 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	FREEZING TREATMENT ON DATE SPECIFIED		
	OCT. 25	NOV. 7	DEC. 6
	−10° C. 2 HOURS	−10° C. 2 HOURS	−20° C. 2 HOURS
Minhardi	725	243	338
Wisconsin Pedigree no. 2	707	309	540
Trumbull	767	527	684

which occurred with cold weather, lower values of electrical conductance are found in exudates from the frozen tissues of varieties known to be hardy than from similar frozen tissues of tenderer strains, where greater injury from freezing occurred. In this particular case, Minhardi, a very frost-resistant wheat, is shown to be hardier than Wisconsin Pedigree no. 2 (a Turkey selection) which in turn is hardier than Trumbull.

EXPERIMENTS WITH OTHER SMALL GRAINS

Several varieties of rye, oats and barley, planted in rows gave results of a very similar nature. Table VI gives an excerpt from abundant data on several dates during the fall. The table shows the considerable degree of difference of the various varieties of rye to withstand severe freezing after all varieties had been exposed to snow and cold weather in the field. The winter and spring varieties of grain were clearly differentiated by a suitable freezing treatment at −5° C., while at −10° C. both were so severely injured that the difference is much less marked. On Oct. 15, very hardy varieties of winter wheat or rye were nearly as much injured at −5° C. as at −10° C. On the contrary, the southern winter varieties had hardened in the warm fall weather to such an extent that they were not damaged to any great degree at −5° C. but were very severely hurt by the more severe freezing treatment. There seems, therefore, to be a decided tendency for the southern winter varieties to harden in milder weather than the very hardy northern strains. An unusually warm period in November brought out the further fact that, with the exception of Minhardi winter wheat, all the ten varieties examined, showed rather definite breaking of the hardened condition with the elevated temperature, and greater injury from freezing following this period.

TABLE VI

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF SMALL GRAIN CROWNS, FROZEN AS INDICATED. AN INTERVAL OF 16 HOURS WAS ALLOWED FOR EXOSMOSIS FROM A 2-GRAM SAMPLE INTO 15 CC. OF DISTILLED WATER AT 0° C.

VARIETY	DATE	FREEZING TREATMENT	SPECIFIC CONDUCTIVITY
Rye			
Wisconsin Pedigree no. 2 winter rye	Nov. 29	2 hours –20° C.	626
Abruzzi winter rye	"	"	769
Italian winter rye	"	"	898
Spring rye	"	"	1024
Oats			
Red rust-proof winter oats	Oct. 15	2 hours –5° C.	333
Wisconsin Pedigree no. 7 spring oats	"	"	665
Red rust-proof winter oats	Oct. 15	2 hours –10° C.	907
Wisconsin Pedigree no. 7 spring oats	"	"	925
Barley			
Union winter barley	Oct. 15	2 hours –5° C.	312
Wisconsin Pedigree no. 38 spring barley	"	"	475
Union winter barley	Oct. 15	2 hours –10° C.	898
Wisconsin Pedigree no. 38 spring barley	"	"	887

ROW VERSUS NATURAL FIELD SPACING

With the small grains planted in thick rows, as with the alfalfa, less satisfactory results were obtained, poorer checks of duplicate samples were secured, and in general a less reliable indication of winter-hardiness could be gained than when the plants were growing in dense, but uniform, populations under natural field conditions. Probably the crowding, uneven covering by soil, snow and surface materials, and the competition for mineral matter contribute to these discrepancies. In the case of alfalfa, the variation in the growth of individual plants in the row was extreme. It does not appear, however, that excessive growth of the seedling plants is necessarily conducive to hardiness and moreover we have clear preliminary evidence that deficiencies, either in regard to mineral nutrition or organic foods, may be restrictive in the full development of the hardened condition. Further investigations of these relations are in progress.

Determination of injury of alfalfa roots by measurement of electrical conductivity of the tissue itself after freezing

In that a test of injury would be useful which involved very small portions of tissue and which would not necessitate sacrifice or even severe disturbance of the mother plant, the following method was devised. One-centimeter sections were cut from various parts of three-year-old roots of Grimm, Utah Common and Hairy Peruvian alfalfa. These sections were quickly weighed, and fastened with fine wire so that they could be suspended in tubes for freezing. The tissues were frozen by suspending the tubes for four hours in a liquid bath at the desired temperature. Thawing was allowed to proceed for several hours in the water-bath at 0° C. After warming to room temperature the root sections were placed between two electrodes actuated by a spring clamp and the electrical resistance of the tissue itself measured. The reciprocal of this resistance gives the conductivity of the section, which is divided by the weight of the section to reduce all values to approximately equal cross-sections and, therefore, comparable values. Preliminary experiments on November 5 had shown that the electrical resistance of the root section decreases very considerably as a result of freezing, and may be, in general, from one-half to one-tenth of its former value. The Grimm sections showed decidedly less change in resistance and conductivity than those from Utah Common alfalfa, while Peruvian sections were still more markedly affected. It had also appeared that pieces of small roots and especially pieces taken at some distance below the crown were more tender than large pieces, especially when taken near the crown. It therefore seemed desirable to investigate the following points in a more extensive experiment.

1. The variation in hardiness of individual plants within a variety as compared with other varieties.
2. Variation in hardiness due to position of the section on the root.
3. Variation due to the size of the root.
4. Comparison of the conductivity of the tissues themselves with values for hardiness obtained by the exosmosis method.

On November 10, eight average, uniform roots and four large roots were selected from each of the three varieties. From each of the 36 roots a two-centimeter section at the crown was removed and retained to be added to the residue of the root for exosmosis. From the first four average roots (1 to 4) of each variety, two one-centimeter sections were cut, adjacent to each other at the upper end of the remaining root. From the other four average roots (5 to 8) the upper one-centimeter section was retained, then six centimeters of root removed below it and another one-centimeter section

secured. The same procedure was followed using two of the larger roots (9 and 10) for adjacent sections, and two (11 and 12) for separated sections. These sections were weighed, suspended by fine wire in moistened tubes, frozen and thawed as previously described. The results are presented in table VII. From this table it is very evident that decided differences existed in the hardiness of individual plants of any given variety on November 10. It is not assumed that these values are a measure of the ultimate hardiness of the individual plants, since they would be expected to vary somewhat in rate of hardening as winter weather approached. It

TABLE VII

VARIATIONS IN ELECTRICAL CONDUCTIVITY OF SECTIONS OF ALFALFA ROOT TISSUES FROZEN 4 HOURS AT −10° C., ON NOVEMBER 10, 1930. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

Root	Position of section	Conductivity ($\times 10^6$) divided by weight of section		
		Grimm	Utah Common	Hairy Peruvian
1	Crown	498	891	1675
	Adjacent	745	972	1755
2	Crown	365	1410	2038
	Adjacent	407	1435	2000
3	Crown	521	618	1960
	Adjacent	617	576	2000
4	Crown	1165	892	4380
	Adjacent	1365	1100	4080
5	Crown	607	1040	1815
	6 cm. below	925	1408	1935
6	Crown	342	1302	1200
	6 cm. below	967	1400	1305
7	Crown	607	1565	1192
	6 cm. below	1209	2050	1485
8	Crown	685	1350	.....
	6 cm. below	1513	1423	.....
9	Crown	192	545	1105
	Adjacent	246	631	1215
10	Crown	697	776	1465
	Adjacent	823	761	1580
11	Crown	358	792	1025
	6 cm. below	598	1338	1510
12	Crown	623	1020	1242
	6 cm. below	1053	1357	1360

is apparent that at this time of year the position of the tissue on the root is of enormous importance in the case of Grimm, of some importance in the case of Utah, and of relatively little importance in the case of Hairy Peruvian roots. The crown tissue of Grimm roots is very much less affected by freezing than the tissue more deeply buried in the soil, while crown tissue of Hairy Peruvian is but slightly different from that at other points on the root. It would appear that the crown tissue of Grimm roots has become hardened by exposure to cold, while that of the tender variety is almost unchanged. The variation due to the size of root is relatively slight, although these data are insufficient to draw definite conclusions. Further data, by this method, later in the season, appear to indicate that the small Grimm roots from field plats are more severely injured by a given freezing treatment. In this test, as in the field, large roots of Utah Common and Hairy Peruvian seem to be distinctly more winter-resistant than smaller roots. Perhaps the small roots are more completely frozen in each case and it may be that the small size of these roots is but a reflection of unfavorable growing conditions which would affect hardiness adversely. It should be remarked that the winter of 1929–30 had killed most of the Hairy Peruvian in the field and that this group of roots no doubt represents the more hardy residue from natural selection. Most of the surviving roots of Hairy Peruvian were very large. The same statements are in a measure true of Utah Common, since over half of the plants in the field were killed during that winter, while the loss of Grimm plants was less than ten per cent. These facts no doubt explain the very great variation in the Grimm roots and the relatively more uniform condition of those of the tenderer varieties. Table VIII summarizes the results presented in table VII as to the range of hardiness of the crowns within each of the three varieties.

TABLE VIII

A SUMMARY OF TABLE VII. RANGE AND AVERAGE OF CONDUCTIVITIES OF ALFALFA CROWN TISSUES AFTER FREEZING 4 HOURS AT −10° C., NOVEMBER 10, 1930

VARIETY	RANGE OF CONDUCTIVITIES OF 12 FROZEN CROWN SECTIONS	AVERAGE OF 12 CROWNS	AVERAGE OF 4 LARGE CROWNS
Grimm	192 to 1165	555	468
Utah Common	545 to 1565	1017	783
Hairy Peruvian	1025 to 4380	1736	1209

Three grams of the residue of each root were frozen and exosmosis allowed to proceed for 20 hours into 15 cc. of water in the usual way. In every case, outstandingly low values of conductivity of the exudate corre-

sponded to low values of conductivity of the tissue itself. Since residues of the roots were used, it was quite impossible to secure samples for exosmosis identical to those used for the measurement of tissue conductivity. In some cases, the sample for exosmosis had a higher position on the root than the one-centimeter sections of which the resistance was measured; in other cases the opposite was true. However, the two methods checked in a satisfactory manner, and outstandingly hardy or tender roots were in all cases indicated by both methods, in spite of the necessary discrepancies in sampling.

Hardening of isolated root sections

It was thought desirable to investigate the artificial hardening of the lower part of the root, since this point might be of great interest in connection with the applied phases of this work. On November 18, 1930, four roots of Grimm alfalfa were cut into twelve sections each, so that each third section in order of position on the root could be frozen and examined without hardening beyond that received in the field. The remaining sections were similarly divided into two groups to be kept at 0° C. for periods of one and two weeks respectively. Thus, sections 1, 4, 7 and 10 from each root were examined without hardening treatment; sections 2, 5, 8 and 11 were kept at 0° C. for one week; while the remaining sections, 3, 6, 9 and 12, were so hardened for two weeks. The sections to be hardened were cut a few millimeters longer than those used directly, so that they could be freshly cut to the proper length just before freezing, thus avoiding drying and formation of scar tissue on the ends of the sections. The sections were frozen and thawed as in the previous experiment. The conductivity values of the tissue at room temperature divided by the weight of the samples are presented in table IX. The test indicates that very positive hardening effects are obtained by keeping isolated sections at 0° C. for a week at this time of year. The effect varies with the individual root to a marked degree, some roots showing greater ability to harden than others. As a result of two weeks of exposure to a temperature of 0° C. two of the four roots withstood a freezing treatment at –12° C. better than they did one at –10° C. previous to hardening. Although the difference between the upper and lower portions of the root is still evident after two weeks of treatment, it is not as prominent as in roots fresh from the field.

In root 1, section 7 gives a peculiar value entirely out of line with any results obtained in the many samples examined. This unusual result may have been due to super-cooling without crystallization, which is relatively common at higher freezing temperatures (as at –5° C.).

An attempt was made to harden alfalfa roots dug August 27. These roots were in a condition of relatively "low organic reserve." In this case,

TABLE IX

SPECIFIC CONDUCTIVITY ($\times 10^6$) OF ONE CENTIMETER SECTIONS OF FOUR GRIMM ALFALFA ROOTS HARDENED AND FROZEN AS INDICATED. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

SECTION	TREATMENT		CONDUCTIVITY			
	HARDENED	FROZEN	ROOT 1	ROOT 2	ROOT 3	ROOT 4
1	Field only	4 hrs. –10° C.	667	560	264	362
4	"	"	943	660	313	588
7	"	"	173	693	362	747
10	"	"	1120	767	562	862
	Additional					
2	1 week 0° C.	4 hrs. –10° C.	529	223	163	323
5	"	"	506	339	185	455
8	"	"	508	372	183	840
11	"	"	540	387	260	483
3	2 weeks 0° C.	4 hrs. –12° C.	634	328	285	646
6	"	"	887	385	251	777
9	"	"	814	392	307	1125
12	"	"	877	438	336	1058

ten 20-gm. samples of roots of each of the three varieties (Grimm, Utah Common and Hairy Peruvian) were prepared and placed in Pyrex tubes which were immersed in water at 0° C. for varying lengths of time. With the examination made by the exosmosis method, after freezing at –10° C. for four hours, very little or no hardening appeared to have taken place in the course of a month of such treatment, with any one of the three varieties. Duplicate samples of the three varieties taken from the hardening bath, were examined on five dates throughout the period, and the last of the "hardened" samples were finally compared on September 26 with samples fresh from the field. The "hardened" tissues were found to be very slightly more tender than the field samples. The peculiarity of failure to harden at this time of year should be examined further. It is quite possible that the attainment of complete hardening is dependent upon late fall conditions, involving such factors as short day, dormant condition, organic storage, and the predominance of sugar rather than starch in the roots.

Summary

Investigation has been devoted to a method previously proposed which indicates that the degree of hardiness of plants of certain species can be determined by measuring the extent of exosmosis of electrolytes from suit-

ably frozen tissue. The method has been applied with satisfactory results to a large group of varieties of alfalfa, to three strains of red clover and to over twenty varieties of small grains. Tissues grown under various conditions have been examined by this method and show the marked influence of environment upon the hardening process.

An alternate method for the determination of frost resistance is presented, in which the electrical conductivity of the tissue itself is determined after freezing. With three varieties of alfalfa of known hardiness, wide varietal differences are shown, as well as marked differences in hardiness of individual plants within a given variety. The upper root tissue of a hardy variety appears to harden first, and prior to winter temperatures is shown to be decidedly more resistant to freezing injury than the more deeply buried parts. But little difference in hardiness of different parts of the root is found in the case of the most tender strain. The hardening of isolated sections of root tissue taken from the field in November is shown to occur in a refrigerator at 0° C. while hardening treatment during rapid regeneration of new growth in August was not successful.

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GROWTH AND DEVELOPMENT AS INFLUENCED BY FRUIT AND SEED FORMATION

A. E. MURNEEK

(WITH THREE FIGURES)

Some years ago BRIGGS, KIDD, and WEST (**3**) summarized the very extensive data which had been accumulated at a much earlier time by U. KREISLER and his co-workers on the growth of several field crop plants, especially *Zea mays* (**14, 15**). The data had been lying more or less dormant and neglected up to that time. When the weekly percentage increases in dry weight (growth rate) and leaf area per unit dry weight (leaf-area ratio) were plotted against time, curves were obtained from these old records which could be roughly reduced to a generalized form (fig. 1).

The similarity in the direction of the two schematic curves thus secured suggests that the growth rate (percentage increase in dry weight) is a function of the leaf area and that it is quite constant during the life cycle of the plant. There are, however, two quite noticeable discrepancies. Growth seems to be independent of the leaf area early in the development of the plant when the seedling is still drawing on the food reserves of the

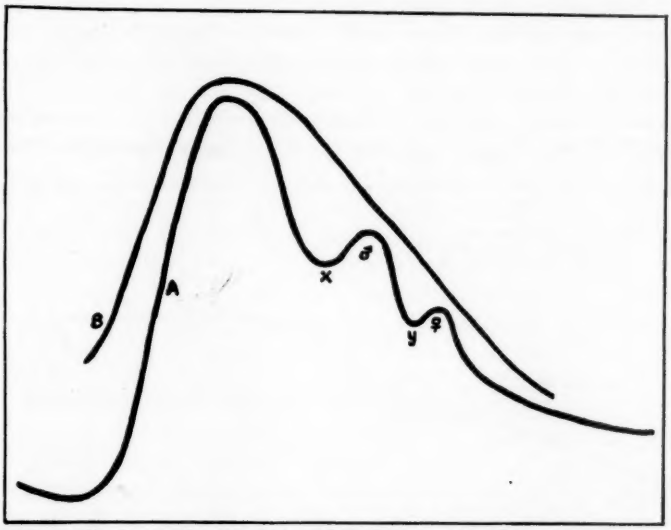

FIG. 1. Generalized growth rate (A) and leaf-area ratio (B) curves for *Zea mays*. After BRIGGS, KIDD, and WEST (3).

seed and while it becomes adjusted to an independent life. The second conspicuous departure of the growth curve is during the period of sexual reproduction of the plant. Since no evident connection between fluctuations in the external environmental factors and alteration in the growth rate during reproduction could be found (**14, 15, 3**), one is forced to conclude, as the authors were, that subsidiary minima (x, y) and maxima (♂, ♀) of the curve are due to internal causes.

It is my object to call your attention to these induced "disturbing" effects on growth and development in plants during the time of sexual reproduction. An attempt will be made to distinguish certain phases in the physiology of reproduction and to correlate these with definite stages in the morphogenesis of flowers and fruits. This discussion will be preceded and accompanied by several references to experimental data.

In his studies with the tomato (*Lycopersicon esculentum*) the writer has demonstrated (**21, 23**) that under all types and with very extreme types of nutrition the presence of a relativity large crop of fruits results in retarded vegetative development. This was found to be particularly true when the plants were subjected to a restricted nitrogen supply. Those on a low plane of nitrogen nutrition were completely inhibited in further growth by a single small fruit. The terminal growing point was often obliterated, the fruit becoming the "tip" of the plant as it were. But a timely removal of the fruit resulted in a complete recovery of vegetativeness and a subsequent normal growth a foot or more in length. On this new portion of the stem flowers again were produced, which, when fertilized, usually set fruit (fig. 2).

A similar situation, only on a larger scale and hence more conspicuous, was exhibited by nitrogen-high plants. These, however, required the pres-

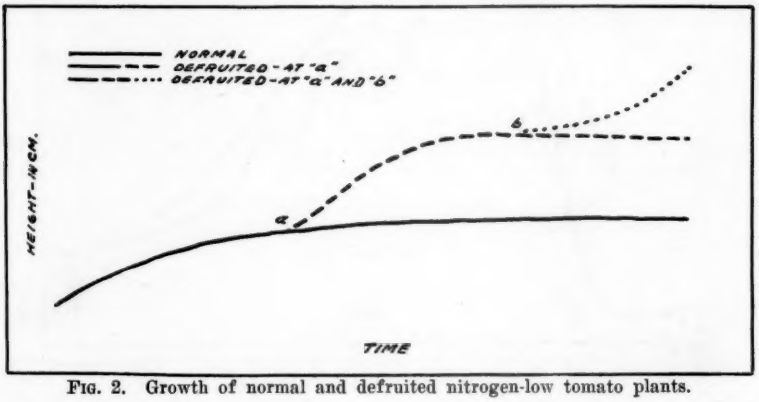

FIG. 2. Growth of normal and defruited nitrogen-low tomato plants.

ence of several large fruits—with extremely vegetative specimens as many as 30—before complete cessation of further vegetative development was brought about. When the fruits were removed from such plants a really extraordinary development took place. Frequently they attained a size twice or three times that of the control plants growing in the same containers.

It has been concluded from this work that the inhibitory effects of the fruit on development proceed in the tomato in approximately the following sequence: (a) Destruction of fecundity of the flowers. Though pollinated the blossoms could not be induced to set fruit. (b) Decrease in size of the flower clusters and all floral organs. (c) Yellowing and abortion of flower buds. (d) Decrease and cessation of further elongation of the stem. (e) Complete exhaustion and eventual death of all parts of the plant, excepting the fruit.

Continuing these investigations in further detail it was found (**24**) that tomato plants absorbed the greatest quantities of soil nutrients and synthesized the largest amounts of organic substances when fertilization (gametic union) was permitted but fruit was not allowed to develop too far. Thus fertilization and the subsequent presence of embryos exercised, during a certain period, a beneficial influence both on the vegetative parts above ground and on the root system. These conclusions are based on chemical analysis of whole plants, treated and normal specimens being grown in an identical environment. Stimulation due to sexual reproduction apparently went beyond the reproductive organs.

These results with the tomato are in complete agreement with those secured with cotton by MASON (**17, 18**), who observed a marked retardation in growth of both the central stem and lateral branches during flowering and fruiting. The rate of elongation of the main axis of the plants decreased in exact proportion to the amount of flowers formed and fruits set. When fruits were present, a conspicuous shedding of the immature flowers was also noted in the cotton. A similar observation had been made somewhat earlier by EWING (**8**), who adds that after the close of the first fruiting season, and when most of the fruits had attained a mature stage, "second growth" is developed resulting in new flowers and fruits. When flowers were removed at the time of shedding, the plants attained a much larger size and the total number of flowers was almost doubled.

Since then my attention has been called to the very interesting studies by EGOROV (**7**) and TARANOVSKY (**28**) on the effects of removal of flowers on growth and absorption of soil nutrients by various field crop plants. These publications had not come to my notice previously, because of interference in exchange in literature incident to the recent war and post-war

unsettled conditions. The records gathered by these authors show that while some plants do not seem to respond greatly to defloration, the majority are affected conspicuously in growth and development. When fruits or seeds were not permitted to develop on these plants the following major physiological and morphological changes were observed: (a) A higher osmotic pressure of cell sap and probably a physical and chemical alteration in cell membranes. (b) A marked increase in development of all vegetative organs, including the roots. (c) A greater assimilation of CO_2, increased absorption of soil nutrients and higher total production of dry matter. The chemical composition of deflorated plants was naturally changed as a result of this treatment, which, for example in buckwheat, was marked by higher percentage concentration and larger total accumulation of phosphorus, potassium, magnesium, chlorine, calcium, and nitrogen. Certain differences in forms of nitrogen, calcium, and other compounds seemed to point not only to an enhanced physiological tempo and better utilization of the environment by the deflorated plants but also to other profound metabolic changes as a response to the removal of sexual organs. Unfortunately it is not stated by the authors at what particular stage of development the flowers were severed from the respective plants.

A partial corroboration of these results, especially those with legumes, may be found in several other contributions on the subject, of which the following may be mentioned: MATTIROLO, (**20**); HECKEL, (**10**); KOTOWSKY, (**13**).

The above review should make it clear that very profound physiological and chemical changes occur in all higher plants during the period of flowering and seed formation. One is thus compelled to share the opinions of several well known students of plant life (BOWER, MOLISCH, MAXIMOW) that in the potential scheme of development or the life cycle of vascular plants the most insistent and effective check seems to be that imposed by the physiological drain of flowering and fruiting.

While we do not know at present, even in a broad outline, the various physiological stages coincident with sexual reproduction, at least two major phases appear to be in evidence and have been emphasized previously by the writer (**24**). In the first place, *as a result of the act of gametic union, and hence immediately following fertilization, there is a marked increase in metabolism at the embryonic point and this increase in a measure extends throughout the body of the plant.* Secondly, *if a maximum number of embryos and their accessory tissues (seeds or fruits) under the particular conditions of nutrition are permitted to develop, the inevitable result will be a retardation in or destruction of further vegetative development, leading under extreme circumstances to death of the plant.*

An ample number of facts accumulated from defloration and other experiments can be marshaled in support of these two physiological phases of reproduction. The declining growth rate and senescence of the vegetative parts of the plant, which usually coincides with the period of embryo development, is conspicuous in many annuals. This stage in the physiology of reproduction does not seem to have received the proper emphasis.

With this consideration in mind, annual plants may be roughly grouped into those with determinate and those with indeterminate type of growth. To the first group belong practically all the cereals, grasses, etc. In these increase in size of the plant usually terminates at the time of fertilization or soon thereafter. The various vegetative structures then undergo rapid senile changes and die promptly. As this event has a strictly seasonal aspect, it thus appears that death of such plants is determined by external factors. In reality, it is due to internal causes which can be readily demonstrated by defloration experiments. Still, what is the mechanism by means of which the vegetative organs are destroyed coincident with fruiting of the plant?

An important feature during this period of development of an annual is the transfer of organic substances from the stem and foliage to the rapidly growing fruits and seeds. This, however, should have no detrimental effect on the metabolism of a plant, since the removal of the end products usually leads to a greater efficiency of physiological processes. Another very conspicuous and most significant change is the onset of yellowing of the leaves and other organs of the plant, resulting most likely from disintegration of the chloroplasts. Apparently the seeds and associated tissues draw not only on the food reserves but also on some necessary substance or substances that constitute a part of the living portion of the cell and are indispensable to the life of the chloroplasts. Thereby the very mechanism of organic synthesis is disrupted or totally destroyed and the organism perforce must die, save for the embryos and tissues closely associated with them.

Naturally, one must assume that such a condition is initiated only when the need of the reproductive organs for certain substances exceeds the current supply. The existing vegetative structures would thus be torn down to make available the necessary ingredients. Analogous instances may be found in the destruction of parental tissues by the animal embryo in case of some important metabolic deficiencies.

Considering this situation and thinking primarily of the soil nutrients which find their way into the seed, EGOROV (7) has expressed the opinion that the real cause of the senescence and death of an annual plant is the loss of magnesium by the chlorophyll. This idea seems to be based on a very abrupt kind of reasoning: Seeds absorb large amounts of the ash ele-

ments from the vegetative structures, including a comparatively high percentage of the total amount of magnesium present in the plant. And since according to WILLSTÄTTER, Mg appears to be the only mineral element present in the chlorophyll molecule, the disruption of the chloroplasts at the time of yellowing of leaves is due to this (assumed) loss of Mg. It is doubtful that this is what actually happens. The disruption of the photosynthetic apparatus does not necessarily require the direct destruction of the chlorophyll molecules. This may happen more likely as a result of the removal of large quantities of some organic substances, such as the nitrogen compounds, from the protoplasm, which is an indispensable part of the living environment for the proper functioning of chlorophyll. Neither can we accept the postulated idea that "the primary cause of the death of an annual that has flowered is the considerable loss of water at the time of flowering, which results in a decrease in assimilatory capacity of the leaves and their gradual dying" (**1**). The loss of water is not the main cause, but a subsequence of some primary influence on protoplasm in the internal physiological readjustment of a plant during sexual reproduction. In many annuals such as the cereals, where flowering lasts but a few hours, and other related processes follow in equally rapid succession, one may easily mistake and substitute one of the more conspicuous effects for the less evident cause.

There is hardly any doubt that large quantities of nitrogen are required for the development of floral structures but especially for the subsequent growth of fruits and seeds. Several investigators (**29, 11, 6, 16**) have shown that at various stages of development the grains of maize, for instance, may contain 50–70 per cent. of the total available nitrogen. Coincident with this concentration of N in the seeds a conspicuous decrease of nitrogen was found to have taken place in the vegetative parts of this plant, especially the leaves. The removal of such large quantities of nitrogen from the leaves to the seeds most likely disrupts the normal structure and function of protoplasm, and eventually leads to loss of water by the leaf and the simultaneous destruction of the chloroplasts. To be sure other indispensable elements of soil nutrition, especially phosphorus and potassium, when *in minimo,* may produce a similar effect. Under ordinary field conditions their shortage, however, is very much less likely to occur, than that of nitrogen.

From the above discussion it is apparent that an antagonism exists between vegetative growth and reproduction, which seems to be especially conspicuous in plants that flower but once, whether these are annuals (*Triticum, Zea,* etc.), biennials (*Beta, Brassica,* etc.), or perennials (*Agava, Corypha,* etc.). The last two groups of plants need not be con-

sidered in detail here, for their behavior in this respect is quite similar to the already discussed annuals. They die promptly after flowering, whether this occurs in the second year or after several years of vegetative growth, the difference being merely a matter of time.

Plants with an indeterminate type of growth, like several of our annuals (*Lycopersicon, Gossypium, Cucurbita,* some legumes, etc.) and most perennial woody plants, do not show a clear cut antagonism between vegetation and reproduction. Both functions usually proceed concurrently. The effects of the correlation between vegetative and reproductive activities, however, are expressed in a more or less clear cut manner here also. Studies with the tomato, the cotton, and certain legumes have been pre-

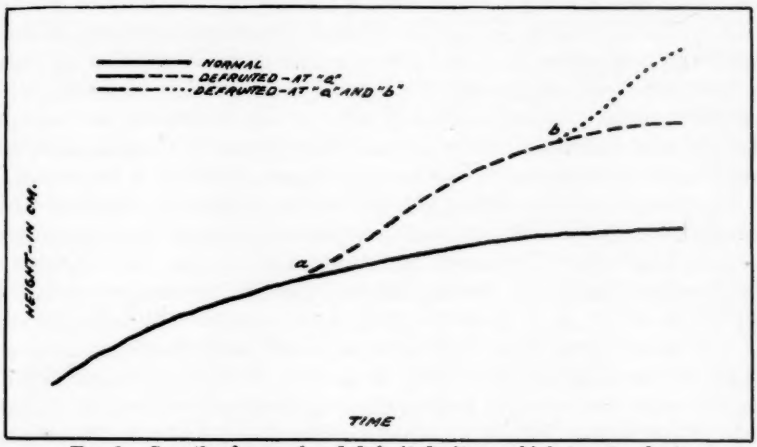

FIG. 3. Growth of normal and defruited nitrogen-high tomato plants.

sented already. In all such plants, vegetative growth usually is curtailed or ceases entirely when a maximum set of fruits or seeds is obtaining. Some of the lower leaves may even be lost in this drain on the food supply by the organs of reproduction. But unless the plant becomes completely exhausted, there is, however, a recovery and resumption of vegetative development after the maturing of the first lot of fruits. Under favorable conditions of nutrition several such recurring cycles of vegetation and reproduction are commonly exhibited by such plants. Moreover, when they become large and branched the effect may become localized to a considerable degree, the fruit drawing on the available food supply primarily in that part of the plant nearest to it.

The lignified perennials present a somewhat difficult situation for analysis. This complexity is due largely to the presence of relatively large

amounts of storage products of diverse type and a pronounced independence of various parts of the tree. In this respect many fruit trees, for instance, should be regarded not so much as individual plants but as rather loose (metabolically speaking) aggregates of independent and competing units (**9**).

That fruiting has a dwarfing influence on the apple tree has been pointed out on several occasions (**9, 22**), but a still more pronounced effect of fruit production seems to be on its subsequent behavior. Heavily bearing trees often do not yield fruit in the following season and may require several years for a complete recovery from an excessive crop. The cyclic flowering and seed bearing of many perennials is a well known phenomenon. This behavior has a direct bearing on the fact that trees utilize for fruit production a large part of the available organic substances, particularly those containing nitrogen. After a year of heavy bearing not only is little stored, but the accumulated reserves of previous years may be heavily depleted. Nitrogen seems to play a very significant rôle in this phase of metabolism of woody plants, especially in the deciduous fruit trees; hence the emphasis on the use of nitrogen fertilizers in the orchard.

Recent studies by the writer (**22, 27**) on the nitrogen and carbohydrate distribution in organs of the bearing apple spur seem to emphasize once more the importance of nitrogen for reproduction. It was found that during flowering and fruit bearing the reproductive organs dominate the metabolism of the spur system and adjoining tissues. Flowering of the apple is characterized by a rapid increase in all active forms of nitrogen, which are moved from other parts of the tree to the flowers and subsequently to the fruits where large quantities eventually accumulate in the seeds. Concomitantly there is a corresponding decrease of N in the storage organs. It has been often observed, likewise, that heavily bearing branches of fruit trees shed their foliage much sooner than branches without or with but few fruits. Thus in this, as in many other respects, trees do not seem to differ essentially from other plants.

Another aspect of the effect of sexual reproduction on metabolism has been pointed out on several occasions and has received at least a cursory consideration. Returning to fig. 1, attention is called once more to the conspicuous departure of the growth rate curve (A) as indicated by the two subsidiary minima (x and y) which precede the time of appearance of the male and female flowers respectively. While some doubt has been expressed (**2**) whether two or only one such depression occurs, also respecting the exact time of appearance of these decreases in growth rate when considered in relationship to the period of flower development, most investigators seem to agree that they are apparent in maize and probably other cereals.

The immediate cause of this depression in accumulation of dry matter, according to some (**2, 3, 1**), is an increased respiration; while others (**2**) believe that it may result from a decreased water content of the assimilatory tissue, especially the leaves. In either case, it is supposed to be of internal origin (**3, 2**). Whatever might be the physiological processes that are instrumental in bringing about this conspicuous decrease in the growth rate, a fundamental initial cause must precede these. The writer ventures the suggestion, hypothetical as it may appear, that if such depressions in growth really occur before anthesis, then they may have some connection with the reduction division of the chromosomes preparatory to spore formation. It must be emphasized most forcibly, however, that this still uncertain effect of sexual reproduction is of an incomparably minor consequence than the aforementioned other two.

A special phase of the writer's investigations in the physiology of reproduction of plants has been an inquiry into the effects of seed development on the subsequent behavior of flowers. The disturbing influence of the presence of fruits on flower performance in the tomato has been referred to already. A favorable material for such a study is the spider flower (*Cleome spinosa*). This vigorous annual produces fruits intermittently. Sterility in this case is due primarily to the periodic abortion of pistils. By submitting a large number of plants to various experimental treatments, it was soon discovered that the developing seeds were responsible for the reduction in growth of the female reproductive organs. Undoubtedly the rapidly growing seeds were able to monopolize the organic food supply to the detriment of the flowers situated immediately above them. A timely removal of the fertilized pistils or young seed pods promptly stopped this drain and, as a consequence, all flowers developed normally and were fertile (**25, 26**).

A somewhat analogous situation was found to exist in the oil palm (*Elaeis guineensis*) (**19**). When young, this plant has two periods of flowering, one in the spring, the other in the fall. As soon as it begins to bear heavily because of age, spring flowering is reduced. This the authors explain as being due to the exhaustion of food reserves by the increased amount of fruits in the autumn, resulting in a shortage of female spadices during the spring period.

These instances tend to show rather clearly that the reproductive organs are able to exert an influence on other parts of the plant, which is somewhat varied in scope but more or less definite in character. The most important feature in the physiology of reproduction seems to be the diversion and localization by the embryos of the products of assimilation.

The question naturally arises as to what may be the means by which the developing embryos are able to draw to themselves and the adjoining

tissues most of the available food supply. To accomplish such results a definite and powerful mechanism certainly must be at the disposal of the reproductive organs. At present, we are entirely ignorant as to its nature beyond the mere conjecture that it may be either of a general physiological or of a specific enzymatic or hormonic character.

In this connection one must emphasize again, though this has been done already on former occasions, that the theory proposed by CHILD (**5**) is worth a serious consideration. Production of gametes may be interpreted as a sign of senescence in plants, associated with a comparatively low rate of metabolism. Fertilization or gametic union, on the contrary, is the beginning of rejuvenescence, initiating high localized levels of metabolism and maintaining them throughout the time of growth of the embryo and its intimate contact with the mother sporophyte. Once a comparatively large number of such metabolic gradients are initiated, as in the case of excessive fecundity, it may lead to diverting of particular substances to the embryo or fruit and the consequent disturbances in the vegetative parts of the plant. The demand to which the sporophyte is exposed by the support of a large crop of seeds and fruits can hardly be overemphasized. Under conditions of comparatively limited absorption of soil nutrients or restricted organic synthesis, the vegetative organs of a plant may become totally exhausted due to the excessive drain by the embryos, leading eventually to death of the whole organism (**12**).

It is probable also that specific stimulating or inhibiting substances, of the nature of enzymes or hormones, may be instrumental in bringing about these effects. They may operate in the general metabolism of a plant as regulating agents. Proof has been accumulating that such catalytic or inhibitory substances do exist in the plant body. It is very likely, therefore, that they are profoundly effective in the metabolism of particular organs during certain stages of development. Such a system of secretions, being endogenous, may be initiated and may come into operation at particular stages of development of the sex organs, but especially during growth of the embryos. The consequent effect will be expressed by a specific alteration in rate or character of development of the vegetative organs or their destruction in plants that support structures devoted primarily to reproduction.

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CHANGES ACCOMPANYING FRUIT DEVELOPMENT IN THE GARDEN PEA[1]

C. S. Bisson and H. A. Jones

(with five figures)

Introduction

Chemical studies on the garden pea were begun at the California Agricultural Experiment Station in 1924. The object of the work was to determine some of the changes that occur in the fruit during development and after harvest. This paper deals specifically with the changes in amounts of some of the components of the pods and peas during their development. The results on storage will be presented in another paper. The investigations were confined mainly to the carbohydrates as it was believed that these played a more important part than other components in the determination of quality.

Experimental procedure

The variety used was Dwarf Telephone. This is a large-podded wrinkled pea and is the variety used for the spring crop in the Imperial Valley. The seed was planted at the University Farm, Davis, California, on November 20, 1924. The plants were in full bloom on April 9, 1925, and on this date a large number of open flowers were labeled by tying strings around the peduncles. This was done to have fruit of the same age for study.

The first picking was made on April 21 when the fruit was 12 days old. The peas at this time were still very small and it was necessary to harvest a large number of fruit to get enough peas for the analyses. A second picking was made on April 27. From this date harvesting was done at two-day intervals until May 15 and then at four-day intervals until May 27. The samples were collected each morning between 7:30 and 8:00. Several representative fruits were selected at each harvest and one specimen of each picking is shown in figure 1. A number of fruits were also counted from each day's picking to be used for samples for analysis. These were then immediately shelled and the peas and pods weighed separately. From these data the average fresh weight of the fruit, pods, and peas were calculated and are given in columns 4, 5, and 7 of table I. When weighings were completed the peas and pods were preserved immediately by placing them

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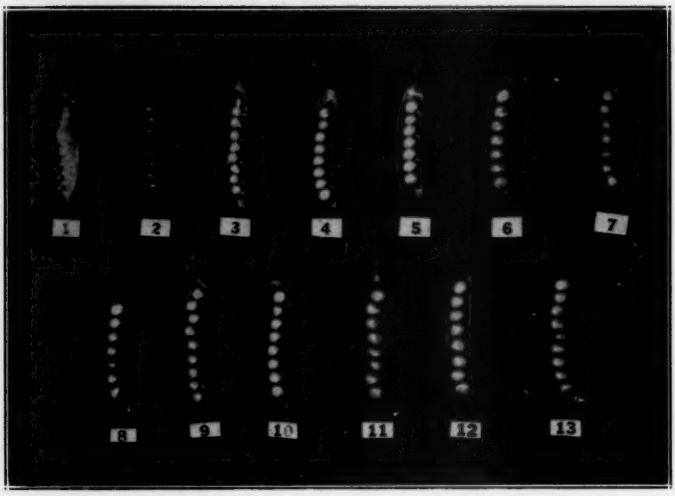

FIG. 1. Fruits at different stages of development. The table accompanying gives the ages in days and date of harvesting.

Figure number	1	2	3	4	5	6	7	8	9	10	11	12	13
Harvested	4–21	4–27	4–29	5–1	5–3	5–5	5–7	5–9	5–11	5–13	5–19	5–23	5–27
Age in days	12	18	20	22	24	26	28	30	32	34	40	44	48

in separate flasks which contained sufficient 95 per cent. alcohol to produce a solution in which the alcohol content was always greater than 65 per cent. by volume after the sample had been added. To the contents of each flask there was added 0.25 gm. of $CaCO_3$; they were then heated to boiling as quickly as possible and held there for 10 minutes. After cooling, the samples were stored. Later they were analyzed for their content of dry matter, total nitrogen, reducing sugars, total sugars, starch, acid hydrolyzable components, crude fiber and ash.

Dry matter.—The stored samples were emptied into large evaporating dishes, and alcohol and water evaporated, and the residues partly dried by slowly heating over a water-bath. In order to prevent the destruction of any of the organic matter, the drying was done between 65 and 70° C. The partially dried samples were then transferred completely to small evaporating dishes and dried in air for 3 days at 65 to 68° C. Drying was completed in a vacuum oven over the same temperature range. After drying 4 days, weighings were made every 24 hours until the loss in weight for the 24-hour period became 0.2 per cent. of the dry residue. The samples

TABLE I

GROWTH RECORDS OF FRUIT AT DIFFERENT STAGES OF GROWTH. VARIETY DWARF TELEPHONE

1	2	3	4	5	6	7	8	9	10	11
DATE HARVESTED (1925)	AGE IN DAYS	NO. OF FRUIT	AV. WEIGHT OF FRUIT	AVEGAGE WEIGHT OF PODS		AVERAGE WEIGHT OF PEAS PER POD		AV. DRY WEIGHT OF FRUIT	FRESH WEIGHT RATIO PODS/PEAS	DRY WEIGHT RATIO PODS/PEAS
				FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT	DRY WEIGHT			
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
4/21	12	150	3.33	3.02	0.37	0.31	0.05	0.42	9.7	7.0
4/27	18	110	4.90	4.04	0.59	0.86	0.14	0.73	4.7	4.3
4/29	20	113	5.11	3.66	0.57	1.45	0.26	0.83	2.52	2.2
5/1	22	116	6.13	4.22	0.61	1.91	0.35	0.96	2.22	1.8
5/3	24	122	6.31	3.98	0.59	2.33	0.47	1.06	1.71	1.3
5/5	26	108	6.65	4.06	0.57	2.59	0.55	1.12	1.57	1.1
5/7	28	101	6.60	3.82	0.54	2.78	0.66	1.20	1.37	0.81
5/9	30	.....	.....	.....	.....	.....	.....	.....	.....	.....
5/11	32	86	7.22	4.00	0.52	3.22	0.82	1.34	1.24	0.64
5/13	34	117	6.21	3.18	0.41	3.03	0.93	1.34	1.05	0.44
5/15	36	118	5.31	2.44	0.36	2.87	0.93	1.29	0.85	0.39
5/19	40	105	5.59	2.42	0.36	3.17	1.09	1.45	0.76	0.33
5/23	44	106	4.80	1.66	0.37	3.14	1.22	1.59	0.53	0.31
5/27	48	139	2.15	0.45	0.36	1.70	1.17	1.53	0.26	0.30

were then ground so that they would pass completely through a 100-mesh sieve, then redried at 65 to 68° C., after which they were placed in bottles and sealed with paraffin.

The dry weights of the residues were corrected for the $CaCO_3$ added. The average dry weights of the pods and peas were calculated from the dry weight and the number of fruits in the sample. The percentages of dry matter in the pods and peas were calculated from the dry and fresh weights of the samples and they are recorded in column 3 of tables II and III. The absolute weight of each component was calculated by multiplying the average dry weight of the sample by the percentage of the component found by analysis.

Total nitrogen.—For the determination of total nitrogen duplicate samples varying from 1 to 2 gm. were used. The procedure given in SCOTT (**5**) was used in making the determinations except that phenol disulphonic acid was substituted for the salicylic acid.

Free-reducing sugars as invert sugar.—Two-gram samples were placed in Gooch crucibles and extracted for 2.5 hours in a Wiley extractor with 75 cc. of 50 per cent. (by weight) alcohol-water solution. The residues were held for starch determinations. The extracts obtained were placed in 250-cc. beakers on a steam-bath and evaporated to about 10 cc., and then completely washed into 200-cc. volumetric flasks with about 150 cc. of distilled water. In order to clarify the solution 3 to 6 cc. of a neutral saturated lead acetate solution were added to each flask; they were then thoroughly shaken and allowed to stand for 15 minutes. Water was then added to the mark; the mixtures were again shaken and then filtered through dry filter papers. Solid $Na_2C_2O_4$ was added to the filtrates in sufficient quantity to precipitate all the excess lead as PbC_2O_4. The mixtures were again filtered and 25- or 50-cc. aliquots were used for the sugar determinations by the method of MUNSON and WALKER (**4**). The Cu_2O was determined by the BISSON-SEWELL (**1**) method.

Total sugars as invert sugar.—Fifty-cc. aliquots of lead-free solutions as prepared above were pipetted into 100-cc. volumetric flasks and then 20 cc. of water and 5 cc. of concentrated hydrochloric acid added; the solutions were heated on a water-bath to 68 to 69° C. in 2.5 to 2.75 minutes, and held at this temperature for a time sufficient to make a total of 10 minutes from the instant heating was started. The solutions were then cooled at once. The solutions in the flasks were neutralized with solid Na_2CO_3 and then diluted to the graduation on the flasks with distilled water. Twenty-five or 50-cc. aliquots of these solutions were used to determine the invert sugars, as described for the determination of reducing sugars. The percentages of free reducing sugars were subtracted from the corresponding

percentages of total sugar, both expressed as invert sugars and the differences multiplied by 0.95 to obtain the percentages of sucrose.

Starch.—The method for determining starch was essentially that of WALTON and COE (**6**).

Total acid hydrolyzable polysaccharides.—The method of determination was essentially the same as that used by BOSWELL (**2**). By deducting the percentage of starch the percentage of acid hydrolyzable polysaccharides other than starch was obtained.

Crude fiber.—The method used for the determination of crude fiber was that given in the official methods of analysis (**4**).

Ash.—Two two-gram samples were weighed into evaporating dishes and ashed in an electric muffle furnace at about 500° C. for 10 minutes. They were then removed from the muffle, cooled and weighed and again heated until a constant weight was obtained.

Experimental results

GROWTH OF PODS AND PEAS

After fertilization, the fruit made a fairly rapid growth. The early increase in weight of the fruit was due almost entirely to the growth of the pods, which reached their maximum weight while the peas were still very small, as shown in table I. When the fruit was 12 days old, the pods had almost reached their maximum size and by the 18th day they were about fully grown. The average *fresh weight* of the pods changed but little from the 18th to the 32nd day, and then there was a gradual decrease. The average *dry weight* of the pods showed a very large increase from the 12th to the 18th day, but little change from the 18th to the 26th day, then there was a gradual decrease until the 36th day and from that time on it remained fairly constant.

As stated above, the peas were still very small when the pods were fully developed. As shown in column 7 of table I the average *fresh weight* of peas per pod increased until about the 32nd day, then they remained fairly constant until the 44th day, after which time there was a rapid decrease due primarily to loss of moisture accompanying ripening. As shown in column 8 of table I there was a gradual increase in the average *dry weight* of peas per pod until the 44th day. This shows that even after they had reached their maximum fresh weight on the 32nd day they still continued to store reserve material until the 44th day.

As shown in table I the growth curves of the pods and peas do not coincide; the data in columns 5 and 7 show that the pods reached their maximum fresh weight about the 18th day and the peas about the 32nd day. There was a gradual increase in the average fresh weight of the entire fruit

until the 32nd day. The increase from the 18th to the 32nd day must have been due entirely to the growth of the peas. There was a rather irregular decrease in the average fresh weight of the entire fruit until the 48th day. Although there was a decrease in the fresh weight over this period, the data in table I column 9 show that there was an increase in the average dry weight of fruit until the 44th day with a slight irregularity from the 32nd to the 36th day.

Chemical composition of the pods

Dry matter.—The results given in column 3 of table II show that in the pods the *percentage* of dry matter remained fairly constant from the 18th to the 40th day after which time there was a rapid increase. Since the average *dry weight* remained fairly constant after the 36th day as shown in column 6 of table I the increase in the percentage of dry weight after the 40th day must have been due to the loss of water accompanying ripening.

Total nitrogen.—The *percentage* of total nitrogen as given in table II column 4 shows a general downward trend from the 12th to the 48th day. However, there were changes in the rate of decrease of percentage over certain periods of this time. From the 12th to the 20th day the decrease was very rapid, from the 20th to the 28th day there was practically no decrease, but after the 28th day there was a decrease again until the 48th day. As shown in figure 2 the *absolute weight* of total nitrogen increased

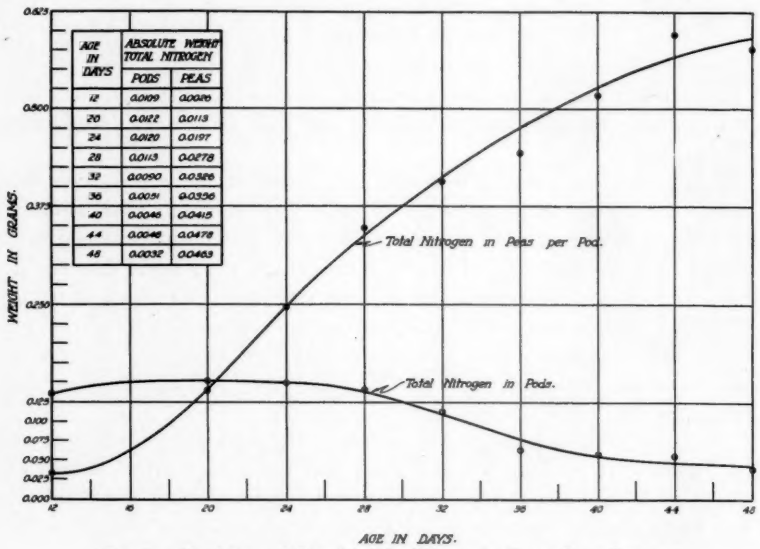

Fig. 2. Absolute weight of total nitrogen in the pods and peas.

slightly from the 12th to the 20th day. It remained practically constant from the 20th to the 26th day; it decreased rapidly until the 36th day, after which time there was but a slight decrease. Since the absolute weight of total nitrogen was calculated from the percentage composition and absolute weight of dry matter, the trend of various portions of the nitrogen curve may be explained as follows: The increase in absolute weight of total nitrogen occurring from the 12th to the 20th day was primarily due to the fact that the rate of accumulation of total dry matter was greater than the rate of decrease in the percentage of nitrogen. From the 20th to the 26th day there was but a slight change in the absolute weight of dry matter and also in the percentage of nitrogen and therefore very little change in the absolute weight of total nitrogen.

Although the absolute weight of total nitrogen remained about constant between the 18th and the 26th day, this does not mean that there was no movement of nitrogen compounds into the pods but rather that the rate of inflow of nitrogen compounds was about equal to the rate of outflow. During this period there was a flow of nitrogen compounds from the stem of the plant through the pods to the peas as indicated by the rapid piling up of these substances in the peas (fig. 2). This shows that the nitrogen compounds entering the pods from the plants are not retained in the pods but are translocated to the peas. From the 26th to the 48th day there was a decrease in absolute weight of total nitrogen. This decrease may be attributed to the fact that nitrogen compounds were moving from the pods to the peas at a more rapid rate than they were entering the pods, thereby causing a drain on the reserve nitrogen compounds in the pods. The rate of drain was greatest during the early part of this period.

Total sugars.—The percentage of total sugars increased until the 24th day when the maximum was reached. From the 24th to the 30th day the percentage remained fairly constant, after which time there was a fairly rapid loss until the 48th day (table II).

The absolute weight of total sugars also showed an increase reaching a maximum on the 24th day, after which time there was a gradual loss. The rate of loss from the 24th to the 36th day was greater than the rate of loss from the 36th to the 48th day (fig. 3). This increase in absolute weight of total sugars was probably due almost entirely to translocation to the peas rather than transformation into other classes of carbohydrates in the pods.

Starch.—It can be seen from figure 3 and table II that there was a slight increase in both the *absolute weight* and *percentage* of starch in the pods from the 12th to the 18th day, after which time there was a gradual decrease until the 34th day. The absolute weight and percentage of starch remained almost constant from the 34th to the 48th day. This showed that there was an early accumulation of starch in the pods which was prob-

TABLE II

COMPOSITION OF PODS AT DIFFERENT STAGES OF GROWTH. AMOUNTS OF CONSTITUENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

1	2	3	4	5	6	7	8	9	10	11
DATE HARVESTED (1925)	AGE	DRY MATTER IN PODS	TOTAL NITROGEN	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE	STARCH	ACID HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)	CRUDE FIBER	ASH
	days	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
4/21	12	12.11	3.00	14.77	20.93	5.85	13.02	10.72	9.92	4.51
4/27	18	14.60	.....	15.03	25.89	10.32	13.39	10.43	11.35	.....
4/29	20	15.62	2.12	14.30	27.64	12.67	11.81	11.75	12.56	4.10
5/1	22	14.45	.....	14.19	27.08	12.24	10.31	11.31	13.05	.....
5/3	24	14.79	2.05	12.75	29.02	15.46	9.05	12.06	14.04	4.32
5/5	26	14.11	.....	14.27	27.28	12.35	8.07	13.18	14.66	.....
5/7	28	14.03	2.11	13.80	28.36	13.83	6.45	.....	14.79	4.67
5/9	30	14.09	.....	13.69	28.30	13.89	6.63	13.26	14.96	.....
5/11	32	13.10	1.72	11.65	23.29	11.06	5.21	15.63	17.62	4.68
5/13	34	12.82	.....	11.37	18.89	7.14	2.20	18.09	20.04	.....
5/15	36	14.77	1.41	8.55	14.19	5.36	2.16	19.22	22.69	6.03
5/19	40	14.76	1.29	7.39	11.64	4.03	0.77	21.61	24.63	6.40
5/23	44	22.61	1.22	3.40	5.95	2.42	2.02	22.26	25.64	6.54
5/27	48	79.07	0.90	1.14	1.89	0.70	2.05	24.32	29.98	6.13

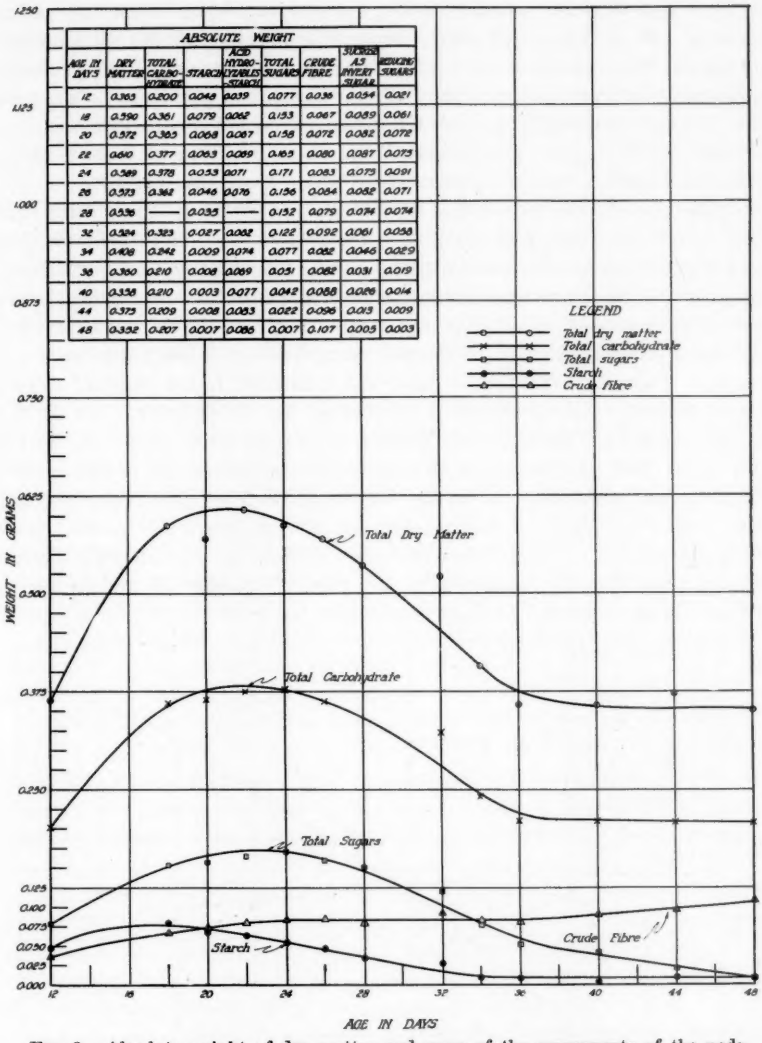

FIG. 3. Absolute weight of dry matter and some of the components of the pods.

ably later hydrolyzed and translocated to the peas. From the 34th day on the starch remained fairly constant at about 2 per cent. of the total dry weight.

Acid hydrolyzable polysaccharides (minus starch).—There was an increase in the percentage of acid hydrolyzable polysaccharides (not including starch) from the 18th day to the 48th day. These compounds probably constitute a large part of the more or less permanent structure of the pod. The increase in percentage of this group of carbohydrates must be accounted for mainly by a decrease in the pods of the more mobile carbohydrates, proteins, and other compounds which move into the peas.

Crude fiber.—The percentage and absolute amount of crude fiber both show a rapid increase from the 12th to the 24th day. The increase in both the percentage and absolute weight of crude fiber during this period can be accounted for by the accumulation of crude fiber at a more rapid rate than the accumulation of total dry matter. From the 24th to the 36th day there was an increase in the *percentage* of crude fiber although not regular. Over the same period there was a decrease in the *absolute weight* of dry matter. This increase in percentage can be accounted for by the loss of constituents from the dry matter other than crude fiber. From the 36th to the 48th day there was an increase in the *percentage* of crude fiber but there was practically no change in the *absolute weight* of dry matter. This caused the absolute weight of crude fiber to show an increase over this latter period. It is possible that the building up of crude fiber during this time was due to a decrease in the rate of transfer of mobile carbohydrates to the peas and to their transformation to crude fiber in the pods.

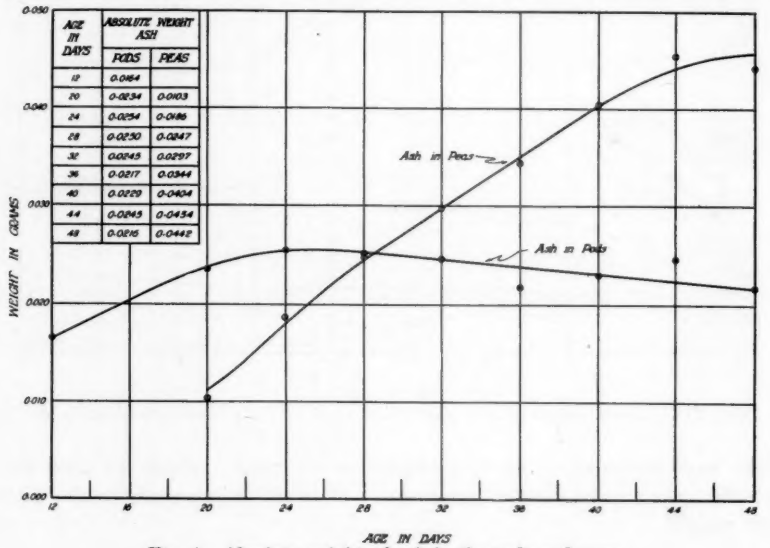

FIG. 4. Absolute weight of ash in the pods and peas.

Ash.—As shown in column 11 of table II there was a considerable decrease in the *percentage* of ash from the 12th to the 20th day. Figure 4, however, shows that there was an increase in the *absolute weight* of ash during this period. The decrease in the *percentage* of ash can be attributed to the fact that the rate of accumulation of mineral constituents in the pods was less than the rate of building up of the organic matter. If these two rates had been equal, the *percentage* of ash would have remained constant. During this period, however, there was an increase in the peas of both ash and organic matter, which showed that there was a continuous flow of these materials from the pods to the peas. From the 24th day on there appears to be a slight decrease in the absolute weight of ash. This indicates that the rate of flow of mineral constituents from the pods to the peas is slightly in excess of the flow from the remainder of the plant to the pods.

Chemical composition of the peas

Dry matter.—The percentage composition of peas at different stages of growth is given in table III. The percentage of dry matter on the 12th day was about the same as on the 18th day. After the 18th day, however, there was a regular increase until the 44th day, after which time there was a very rapid increase due to the loss of water accompanying ripening. As shown in figure 5 the rate of increase in dry weight of the peas from the 12th to the 18th day was less than the rate from the 18th to the 44th day. During the early part of this latter period the rate of increase was almost constant. The average dry weight of the peas reached a maximum at about the 44th day.

Total nitrogen.—As shown in column 4 of table III there was a gradual decrease in *percentage* of nitrogen from the 12th to the 40th day, after which time there was a slight increase. This same trend in the percentage of total nitrogen was also obtained by Boswell (**3**) in his studies with the variety Alaska. As shown by data in figure 2 there was a gradual increase in *absolute weight* of total nitrogen from the 12th to the 44th day. The decrease in the *percentage* of total nitrogen to the 40th day may be attributed to the fact that the rate of increase in the weight of dry matter was many times greater than the rate of increase in absolute weight of nitrogen.

Sugars.—The amounts of reducing sugars in the samples collected from the 20th to the 40th day were too small to be determined.

As shown in column 7 of table III the percentage of sucrose increased from the 12th to the 22nd day. The rate of accumulation of sucrose during this period was greater than the rate of accumulation of total dry weight, which accounts for the increase in percentage of sucrose. From the 22nd to the 44th day there was a considerable decrease in the percentage of sucrose. This may be attributed to the fact that the rate of accumulation

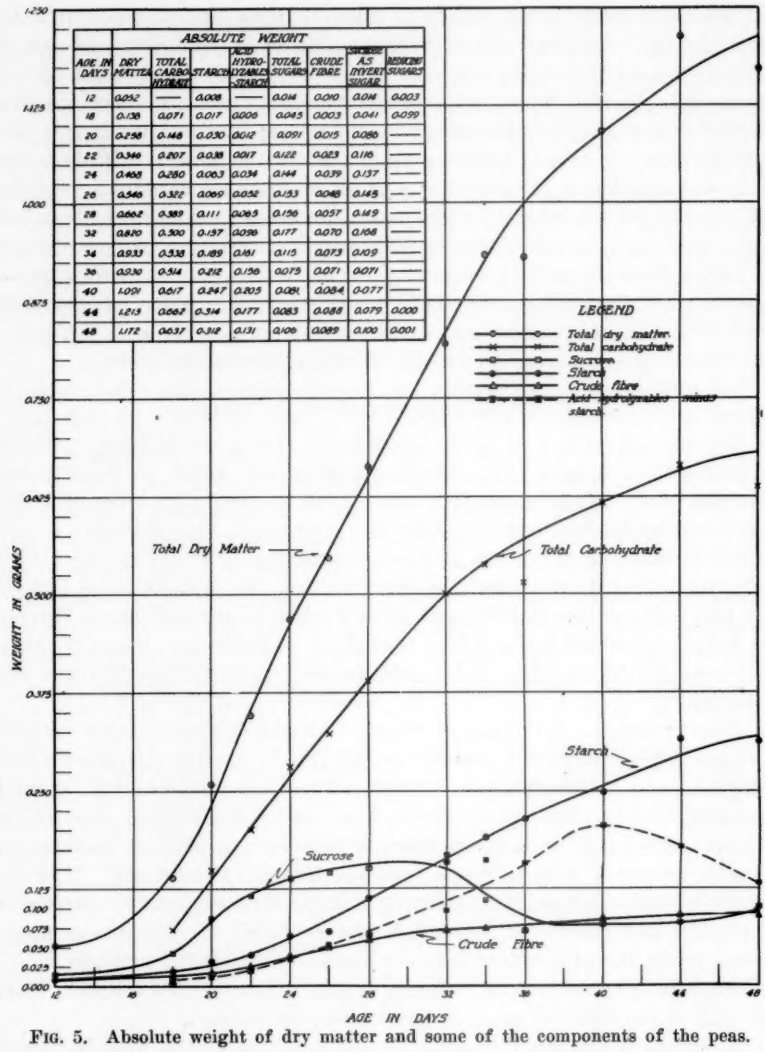

FIG. 5. Absolute weight of dry matter and some of the components of the peas.

of total dry matter together with the rate of transformation of sucrose into other carbohydrates was greater than the rate of accumulation of sucrose.

As shown in figure 5 there was a continuous increase in absolute weight of sucrose until the 32nd day; there was then a decrease until the 40th day, after which time it remained fairly constant. While the maximum *per-*

TABLE III

COMPOSITION OF SHELLED PEAS AT DIFFERENT STAGES OF GROWTH. AMOUNTS OF CONSTITUENTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

1	2	3	4	5	6	7	8	9	10	11
DATE HARVESTED (1925)	AGE	DRY MATTER IN PEAS	TOTAL NITROGEN	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE	STARCH	ACID HYDROLYZABLE POLYSACCHARIDES (STARCH DEDUCTED)	CRUDE FIBER	ASH
	days	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
4/21	12	16.66	5.16	0.53	27.74	25.84	16.33	.....	1.93	.....
4/27	18	15.99	.....	0.66	33.00	30.70	12.49	4.27	2.15	.....
4/29	20	17.76	4.36	.....	35.10	33.33	11.81	4.75	5.78	3.98
5/1	22	18.17	.....	.....	35.34	33.57	10.97	5.04	6.77	.....
5/3	24	20.09	4.22	.....	30.71	29.17	13.43	7.33	8.40	3.89
5/5	26	21.05	.....	.....	28.04	26.64	12.60	9.53	8.85	.....
5/7	28	23.84	4.20	.....	23.58	22.40	16.70	9.85	8.52	3.73
5/9	30	23.50	.....	.....	23.86	22.67	16.06	10.87	8.37	3.26
5/11	32	25.51	3.98	.....	21.55	20.47	19.13	11.71	8.55	3.62
5/13	34	30.77	.....	.....	12.28	11.67	20.22	17.25	7.78	.....
5/15	36	32.40	3.83	.....	8.07	7.67	22.83	16.79	7.66	3.70
5/19	40	34.41	3.80	.....	7.39	7.02	22.67	18.80	7.69	3.70
5/23	44	38.71	3.93	0.04	6.86	6.48	25.84	14.55	7.31	3.74
5/27	48	68.80	3.95	0.09	9.10	8.56	26.63	11.15	7.62	3.77

centage of sucrose was reached on the 22nd day the *absolute weight* of sucrose did not reach a maximum until about the 30th day, at which time the peas were in the proper stage of development for harvest. This was about 8 days after they had reached their highest *percentage* of sucrose, but at this time the *absolute weight* of sucrose in the peas was at its maximum.

Starch.—As shown in column 8 of table III there was a decrease in *percentage* of starch from the 12th to the 22nd day, after which time there was an increase until the 48th day. The *absolute weight* of starch showed a continual increase from the 12th to about the 44th day (fig. 5).

Acid hydrolyzable polysaccharides (minus starch).—The *percentage* and *absolute weight* of acid hydrolyzable carbohydrates other than starch increased from the 18th to about the 40th day, after which time there was a decrease (table III and fig. 5).

Crude fiber.—As shown in table III there was a very rapid increase in the *percentage* of crude fiber from the 18th to about the 24th day. From about the 24th day to the 48th day the *percentage* of crude fiber remained fairly constant. There was a fairly regular increase, however, in the *absolute weight* of crude fiber from the 18th to the 48th day. The increase in the absolute weight of crude fiber during the time the peas were increasing in size can be accounted for mainly by the increase in the number of cells. After the peas had reached their maximum size the increase in crude fiber was probably due mainly to the thickening of the cell walls. After about the 24th day the *percentage* of crude fiber remained about constant but the *absolute weight* of crude fiber continued to increase. This means that the rate of increase in crude fiber was about equal to the rate of increase in total dry matter of the peas; this phenomenon would cause the percentage to remain constant.

Ash.—From the 20th to the 30th day there was a decrease in the *percentage* of ash (table III, column 11). From the 30th to the 48th day the *percentage* of ash increased slightly. The *absolute weight* of ash (fig. 4) showed a regular increase from the 20th to the 44th day. The early decrease in percentage of ash was due to the fact that the organic matter was accumulating at a more rapid rate than the ash constituents. The small increase in percentage of ash during the latter period, if significant at all, can be attributed to the fact that the rate of accumulation of ash constituents was slightly in excess of that of the organic matter.

Summary

1. The weight of the fresh fruit increased up to about the 32nd day, after which time there was a decrease due to the loss of moisture taking place at a more rapid rate than the increase in dry matter. The average

dry weight of the fruit increased throughout the entire period. The increase in dry weight of the fruit up to the 22nd day was due to the increase in weight of both pods and peas, but after the 22nd day the increase in dry weight of the fruit was due entirely to the growth of the peas.

2. In the pods there was an early maximum reached in the absolute weights of nitrogen, total sugars, starch and ash, after which there was a decline. There was a general upward trend in absolute weights of acid hydrolyzable polysaccharides other than starch and in crude fiber.

3. In the peas there was an increase in absolute weight of nitrogen, starch, crude fiber and ash throughout the entire growth period. The absolute weight of sucrose reached its maximum on about the 32nd day and then fell off rapidly. The acid hydrolyzable polysaccharides other than starch reached their maximum weight toward the latter part of the development period.

4. The crude fiber was the only constituent that showed an upward trend in absolute weight throughout the entire period in both pods and peas. The absolute weight of the acid hydrolyzable polysaccharides showed an upward trend throughout the entire period for the pods and for almost the entire period for the peas.

5. Sucrose is probably the most important constituent determining sweetness in the garden pea. In this experiment when this substance was highest in percentage the peas were still too small to be harvested. They were not at the best size for harvesting when the *percentage* of sucrose was at its maximum but they were ready for harvest at the time when the *total weight* of sucrose was at its highest.

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SEASONAL CHANGES IN THE COMPOSITION OF THE NON-PROTEIN NITROGEN IN THE CURRENT YEAR'S SHOOTS OF BARTLETT PEAR

A. S. MULAY

(WITH SIX FIGURES)

It has been shown in a previous paper (**4**) that there are significant changes in the quantity of the non-protein nitrogen in the Bartlett pear shoots during their seasonal growth. It was interesting to know whether the quality of this nitrogen was also changed with the season. Non-protein nitrogen, therefore, was subjected to further fractionation.

Material and methods

The material used was the non-protein nitrogen filtrate, the seasonal changes in which had been studied in a previous paper (**4**). This non-protein nitrogen filtrate, obtained by extracting the samples with water and then precipitating the proteins from the extract, was fractionated before and after hydrolysis into amide, amino, basic, humin, rest, and melanin fractions by the modified VAN SLYKE method (**3**). The chart on page 116 gives a schematic picture of the complete fractionation procedure.

UNHYDROLYZED

Amide nitrogen.—This fraction was determined on an aliquot (C_2)[1] of the filtrate (C) by SACHSSE's (1873) method. Enough HCl was added to the aliquot to make a 4 per cent. HCl solution. The acidulated solution was placed in a boiling water-bath under a reflux condenser for two hours. The hydrolysate was made nearly neutral with sodium hydroxide solution and alkaline with heavy MgO. The resulting ammonia was distilled over into 0.02 N acid and titrated with 0.02 N alkali. This includes both ammonia (*i.e.*, nitrogen from ammonium salts) and amide nitrogen.

Humin nitrogen.—The alkaline mixture remaining from the amide distillation was filtered and the residue (E) washed until free of chlorides, and discarded. Combined filtrate and washings (D) were acidified with HCl, and concentrated under diminished pressure. The concentrate was diluted and aliquots (D_1) taken for nitrogen determination. Difference between the value for N.P.N. (non-protein nitrogen) (C_1) and that for amide N (C_2) plus filtrate nitrogen (D_1) gives the value for (E) humin nitrogen in the residue, *i.e.*, humin nitrogen (E) = $(C_1) - [(C_2) + (D_1)]$.

Basic nitrogen.—From the remainder of the filtrate (D) basic nitrogen was removed by phosphotungstic acid precipitation as described by MOR-

[1] Letters in parenthesis refer to the chart.

TABLE I

NON-PROTEIN NITROGEN FRACTIONATION EXPRESSED AS PERCENTAGE OF TOTAL NON-PROTEIN NITROGEN

BARK

DATE COLLECTED	MELANIN	AMIDE		HUMIN		BASIC		AMINO		REST	
		UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Sept. 16	2.3	14.8	20.5	33.4	17.5	12.3	24.8	24.2	26.0	15.2	8.9
Oct. 21	4.1	10.7	16.0	31.4	10.4	19.2	30.6	23.6	24.5	14.6	14.5
Dec. 7	3.2	13.2	16.6	20.2	8.8	25.1	31.0	16.0	24.4	25.4	15.9
Jan. 15	11.5	10.6	13.1	26.5	11.5	32.8	31.6	9.9	.....	20.1	.....
Feb. 11	9.4	11.6	14.3	22.9	8.8	33.5	27.2	15.3	.....	16.6	.....
Mar. 18	9.9	13.8	16.3	26.3	8.1	30.9	29.9	18.5	24.1	10.8	11.6
Apr. 18	10.3	25.4	28.2	18.9	9.3	19.2	7.9	32.8	33.0	3.6	11.3
May 13	1.0	27.4	31.4	19.2	20.4	15.4	1.0	34.3	37.4	3.4	9.4
June 24	.....	27.0	31.2	23.0	17.7	15.1	5.4	35.9	35.9	0.4	9.9
July 15	4.7	20.6	25.8	39.0	11.4	8.7	11.6	27.6	35.8	4.1	10.6
Aug. 19	6.5	16.5	21.3	36.2	11.9	13.4	10.2	28.0	30.9	5.3	19.1
Sept. 19	4.0	13.1	18.9	32.7	10.1	20.7	21.4	25.2	29.2	8.1	16.3
Oct. 15	5.8	13.8	18.4	31.9	6.7	22.2	29.5	20.1	25.7	11.7	13.8
Nov. 17	7.5	12.3	16.7	30.7	8.6	14.4	27.4	16.2	21.9	25.5	17.7
Jan. 18	7.9	11.9	17.0	35.9	6.4	13.1	21.7	19.3	25.6	19.8	20.4

ROW (3). The filtrate (F) thus obtained was analyzed for nitrogen (F_1). The difference between the nitrogen values (D_1) and (F_1) was taken as basic nitrogen (G).

Amino nitrogen.—This fraction was determined on filtrate (F) by VAN SLYKE'S method as described by MORROW. For these determinations a micro-burette was used in conjunction with a macro-deaminizing vessel of the VAN SLYKE apparatus. As the samples analyzed had no foaming tendency, caprylic alcohol was not used.

Rest nitrogen.—This fraction, also referred to in the literature as other nitrogen, was determined by the difference between nitrogen (F_1) in phosphotungstic acid filtrate (F) and amino nitrogen (F_2).

HYDROLYZED

The remainder of the filtrate (C) obtained by the removal of soluble proteins from the extract was concentrated *in vacuo* to 100 cc. and hydrolyzed with 20 per cent. HCl. The hydrolysate was filtered, the residue (I) washed until free from chlorides, and discarded. The filtrate and washings (H) were evaporated nearly to dryness under reduced pressure, and the residue was taken up with hot water and made up to volume.

Melanin nitrogen (Mel. N.), also referred to as acid insoluble humin in the literature, was determined by the difference between nitrogen value (H_1) on an aliquot of the filtrate (H) and that of N.P.N. (C_1).

Amide, humin, basic, amino, and rest nitrogen was determined as described for the unhydrolyzed sample.

Results

Tables I and II give the results of fractionation before and after hydrolysis of non-protein nitrogen of bark and wood into amide, amino, basic, humin, rest, and melanin nitrogen. The seasonal changes in the composition of non-protein nitrogen in the bark and the wood can be better seen from figures 1 and 2. Figures 3 and 4 bring out the changes resulting from hydrolysis in distribution of nitrogen among different fractions of non-protein nitrogen in bark, and figures 5 and 6 show the same for wood.

Discussion

Total non-protein nitrogen which forms about 50–80 per cent. of the total soluble nitrogen in the bark and 80–90 per cent. of it in the wood was further fractionated into amide, humin, basic, amino, and rest nitrogen before and after hydrolysis.

Amide and amino nitrogen.—The bark in 1927 shows a drop in amino nitrogen from 24 per cent. of non-protein nitrogen in September to about 10 per cent. in January (fig. 1) while the amide nitrogen keeps its level of

TABLE II

NON-PROTEIN NITROGEN FRACTIONATION EXPRESSED AS PERCENTAGE OF TOTAL NON-PROTEIN NITROGEN

WOOD

DATE COLLECTED	MELANIN	AMIDE		HUMIN		BASIC		AMINO		REST	
		UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED	UNHYDROLYZED	HYDROLYZED
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Sept. 16	2.8	13.8	15.1	7.7	14.5	18.1	37.1	17.0	24.3	43.3	6.4
Oct. 21	1.4	5.4	8.4	12.9	12.3	34.7	50.5	9.7	13.7	40.6	13.8
Dec. 7	9.1	6.2	8.6	9.7	10.8	32.5	41.0	10.3	15.2	41.5	15.3
Jan. 15	5.6	5.4	6.3	3.0	9.7	42.9	50.5	11.2	14.7	37.4	13.2
Feb. 11	3.1	5.5	6.6	1.0	8.9	49.3	51.8	7.1	14.3	37.2	15.4
Mar. 18	5.2	5.7	6.7	2.0	8.5	51.8	55.3	8.3	9.5	32.2	14.8
Apr. 18	2.9	24.8	17.5	.3	4.6	31.3	46.4	14.8	6.3	28.6	22.4
May 13	4.1	37.0	27.9	.....	1.4	18.9	24.3	21.8	16.7	22.6	25.4
June 24	1.6	32.4	30.7	1.8	4.4	21.0	25.1	22.7	23.9	22.0	14.2
July 15	2.5	23.0	22.2	3.0	2.0	38.1	44.8	16.1	15.2	20.2	13.4
Aug. 19	3.2	15.8	13.7	1.7	4.3	53.4	56.5	11.6	6.3	17.4	16.1
Sept. 19	7.8	12.6	9.9	2.2	1.6	59.0	58.8	8.9	7.6	17.3	14.3
Oct. 15	4.1	7.0	7.7	4.3	5.2	68.2	71.1	6.2	6.7	14.3	5.3
Nov. 17	1.3	6.2	7.7	4.6	3.1	65.7	75.4	5.8	6.5	17.7	5.9
Jan. 18	0.7	5.7	6.9	6.2	8.2	63.6	72.2	6.6	6.3	19.0	5.8

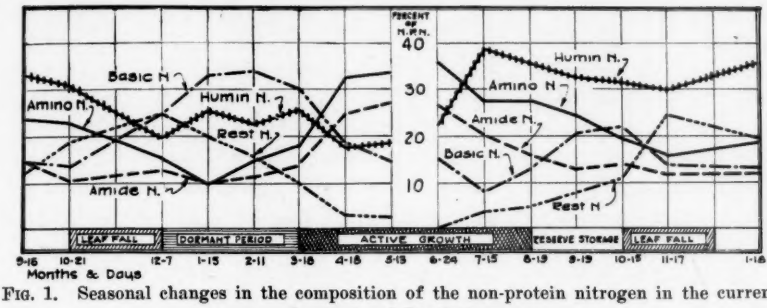


FIG. 1. Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear.

about 12 per cent. during all this time. In February both amino and amide nitrogen begin to increase and by May reach, respectively, the level of 34 and 28 per cent. of the non-protein nitrogen. The bark of 1928 shoots has high amino and amide concentration in June (36 and 27 per cent. respectively) which falls off steadily during the growing season and reaches a low level of 16 and 12 per cent., respectively, in November.

Amide and amino nitrogen in the 1927 wood (fig. 2) keep a low level of about 6 and 10 per cent., respectively, all through autumn and winter

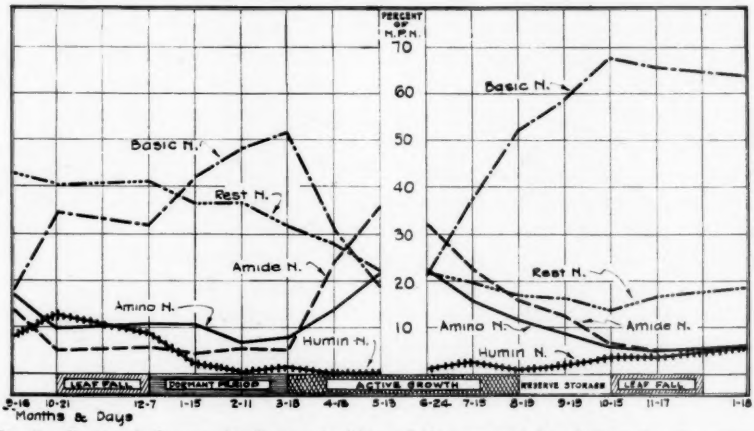


FIG. 2. Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear. (Wood, unhydrolyzed.)

and begin to rise in March. Amide nitrogen increases at a faster rate than the amino fraction and as a result by May the amide fraction is over one and one-half times as large as the amino fraction. In the 1928 shoots, the wood has about 32 per cent. amide and 23 per cent. amino nitrogen in June

which falls during the growing season and reaches a low level for both of about 6 per cent. in November.

Shoot bark and wood are high in amino and amide nitrogen in May and June and decrease until they are at their lowest in winter. In the bark they begin to increase in February, sometime before the buds begin to swell, while in the wood they increase after the trees have come into full bloom. In the bark, amide nitrogen is always at a lower level than amino nitrogen while in the wood all through the active growing period amide nitrogen is decidedly higher than the amino fraction.

The general parallelism here reported between the changes in amide and amino nitrogen in both bark and wood do not agree with the results of SURE and TOTTINGHAM (**6**), NIGHTINGALE (**5**), and THOMAS (**7**). These workers find that the changes in amide and amino nitrogen take place in opposite directions. This may be due to differences in the material used, differences in experimental conditions, or both.

Basic nitrogen.—In the 1927 bark (fig. 1) basic nitrogen increases all through autumn and winter, attaining a maximum of about 34 per cent. in February and then falling to about 14 per cent. in May. In the 1928 bark it falls from 15 per cent. in June to 8 per cent. in July and then rises steadily to 22 per cent. in October, falls to 14 per cent. in November and remains there until January.

Basic nitrogen in the 1927 wood (fig. 2), similar to that in the bark, increases all through autumn and winter, attaining its maximum of 52 per cent. in March and then falls sharply to about 19 per cent. in May. The wood of 1928 has 22 per cent. basic nitrogen in June which rapidly increases to 68 per cent. in October and then changes but little until January.

The basic nitrogen constitutes a much higher fraction of non-protein nitrogen in the wood than in the bark. Both wood and bark start growth with a high concentration of basic nitrogen which falls in the first part of the active growth period and rises during the latter part. It continues to increase all through the dormant period. It begins to fall in the bark when the buds open and in the wood a month later. Fluctuations in basic nitrogen run in directions opposite to those in amide and amino nitrogen.

Humin nitrogen.—The humin nitrogen in the 1927 bark (fig. 1) falls from 33 to 20 per cent. of the non-protein nitrogen between September and December and remains constant the remainder of the season. In the 1928 bark it shows a sharp rise from 23 per cent. in June to 39 per cent. in July, which gradually falls to 30 per cent. in November and then rises to 36 per cent. in January.

The humin nitrogen in the 1927 wood falls from about 10 per cent. in September to about 1 per cent. in February, where it stays until May. In the 1928 wood it starts at about 1 per cent. in June and reaches about 6 per cent. by January.

Rest nitrogen.—In the 1927 bark rest nitrogen increases until December (fig. 1) when it is 23 per cent. of the non-protein nitrogen, then it steadily falls to 3 per cent. by April. The bark of 1928 has no rest nitrogen in June but it accumulates to 25 per cent. by November, after which it begins to fall.

Rest nitrogen in the 1927 wood (fig. 2) remains at 40 per cent. of the non-protein nitrogen from September to December, then falls and reaches a lower level of about 22 per cent. in May. In 1928 wood rest nitrogen shows a gradual drop from 22 per cent. in June to 14 per cent. in August and then a slow rise to 19 per cent. in January.

From the preceding discussion of the non-protein nitrogen fractions of bark and wood it appears that in June, when the first samples are taken from new growth, both bark and wood start with high amide and amino nitrogen which gradually fall, reach the lowest points after the growth in length stops, and begin to rise again when buds are swelling and opening. Basic nitrogen both in the bark and in the wood is at a low level when the first samples are taken from the new growth, reaches its maximum some time before the next year's buds begin to swell and falls sharply after the buds begin to open. In the bark, humin nitrogen increases sharply in the latter part of the growing season and then keeps at that level the remain-

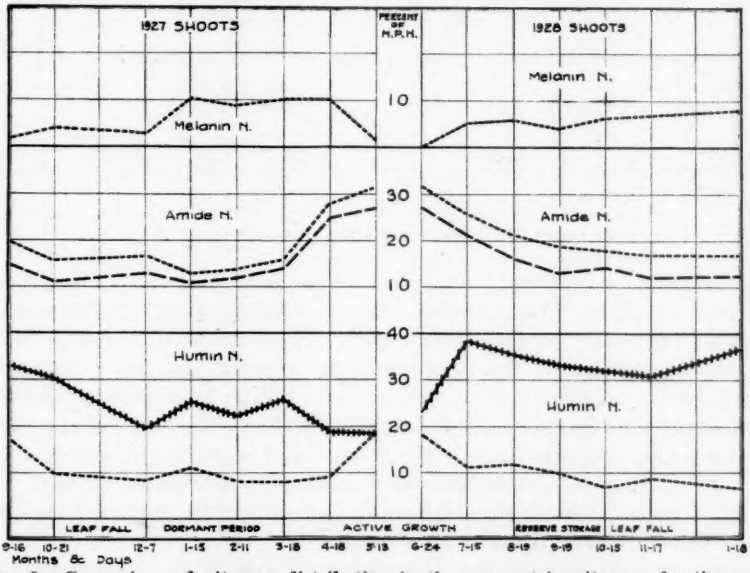


FIG. 3. Comparison of nitrogen distribution in the non-protein nitrogen fractions of bark before (solid line) and after (dotted line) hydrolysis.

der of the season, while in the wood it rises gradually after length growth stops and falls to a low level when the buds begin to swell. Rest nitrogen in the bark increases from June to December while that in the wood increases from August to December and it falls in both from January to May.

Effects of hydrolysis.—On comparing values obtained for different non-protein nitrogen fractions of bark before and after hydrolysis (figs. 3 and 4) it is seen that after hydrolysis the humin fraction diminishes and there

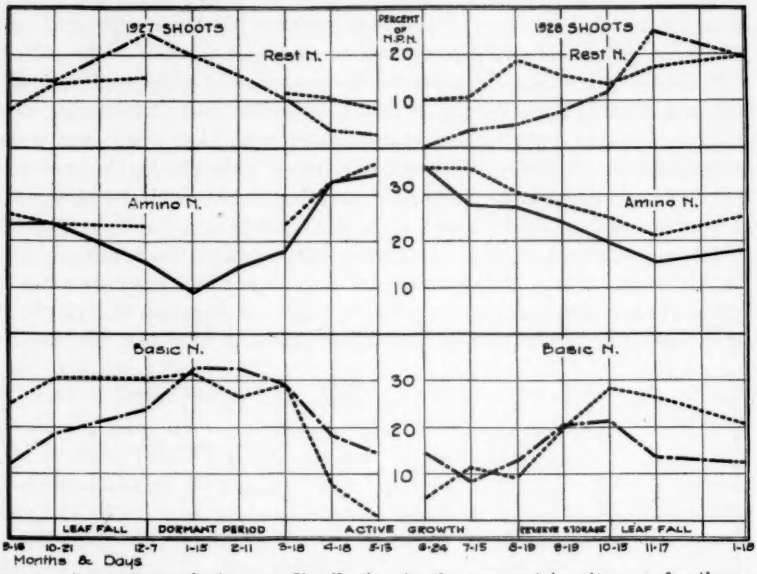


FIG. 4. Comparison of nitrogen distribution in the non-protein nitrogen fractions of bark before (solid line) and after (dotted line) hydrolysis.

is a concurrent increase in amino and amide nitrogen. Basic nitrogen increases in autumn and in winter when trees are dormant and decreases in spring when active growth is beginning. Rest nitrogen, on the other hand, increases during the active growing season and tends to decrease in autumn and winter. A new fraction, melanin, is formed by hydrolysis which takes its nitrogen from the amide and amino fractions. Tryptophane probably plays a most important rôle in the formation of melanin nitrogen (**2**).

The humin fraction, which is most affected by hydrolysis, seems to consist of polypeptids which on hydrolysis break up into simpler forms and are distributed into different fractions.

In the wood, rest nitrogen decreases on hydrolysis with a concurrent increase in basic nitrogen (figs. 5 and 6). This effect is most marked in the

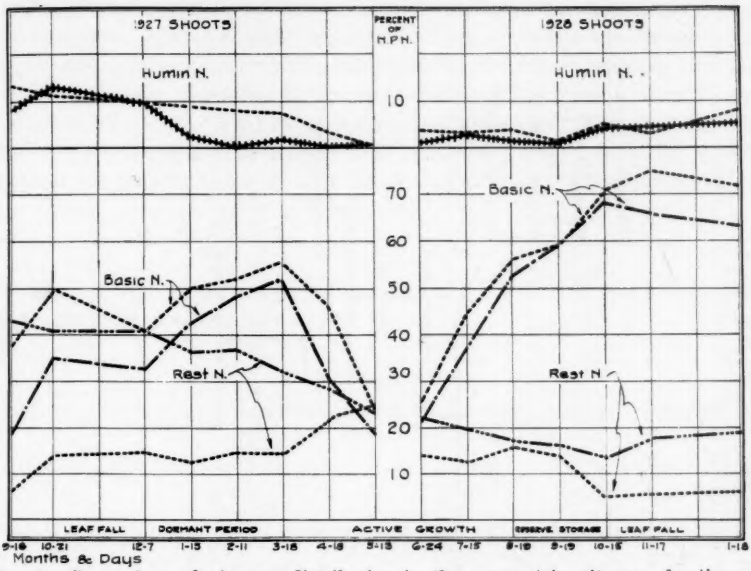


FIG. 5. Comparison of nitrogen distribution in the non-protein nitrogen fractions of wood before (solid line) and after (dotted line) hydrolysis.

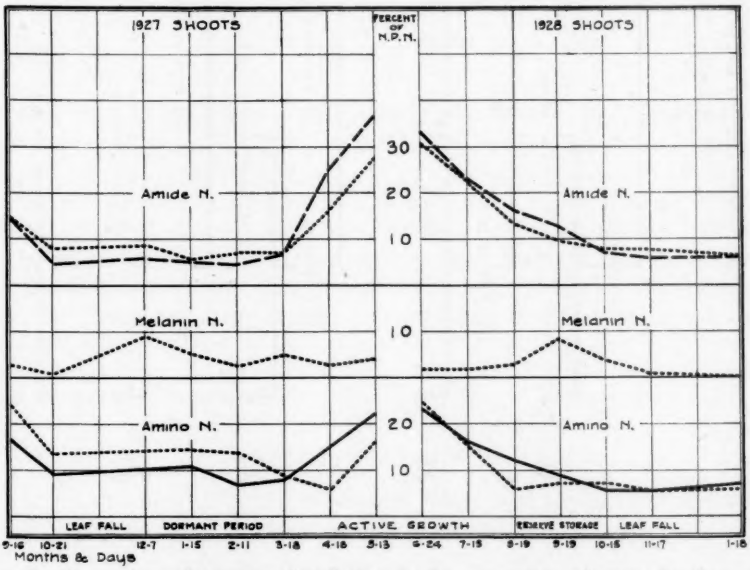
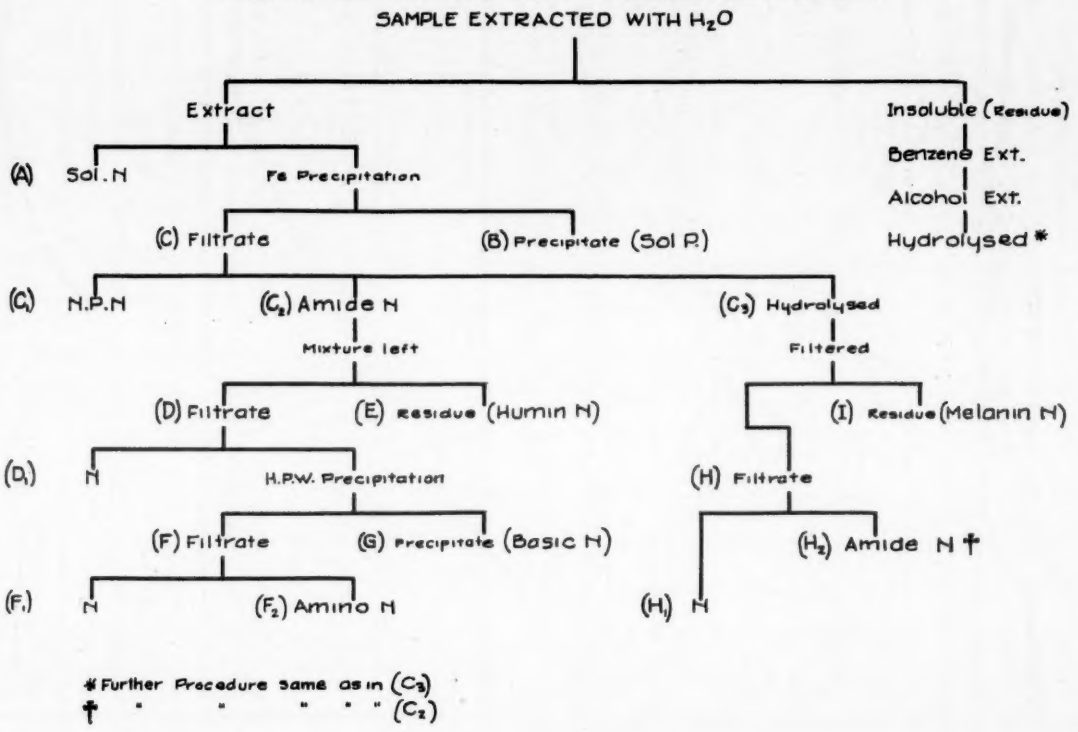


FIG. 6. Comparison of nitrogen distribution in the non-protein nitrogen fractions of wood before (solid line) and after (dotted line) hydrolysis.

PLAN OF NITROGEN FRACTIONATION



1927 wood where basic, amino, and amide nitrogen increase at the cost of rest nitrogen. CHIBNALL (1) has found in his starvation experiments that hydrolysis decomposes the rest nitrogen compounds and suggests that this fraction may be ureide-like in nature. THOMAS (7) suggests peptid combinations, not precipitated by phosphotungstic acid, and VICKERY (8) finds that some polypeptids in alfalfa plant juice are precipitable by this reagent. Carbohydrate amino acid compounds may be suggested here as another form of hydrolyzable rest nitrogen.

Summary

The distribution of non-protein nitrogen in different fractions and their fluctuations is summarized in table III.

TABLE III

RANGE OF FLUCTUATIONS IN NON-PROTEIN NITROGEN FRACTIONS, EXPRESSED AS PERCENTAGE OF NON-PROTEIN NITROGEN

MATERIAL	AMIDE	HUMIN	BASIC	AMINO	REST
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Bark 1927	10–28	24	12–32	10–30	3–23
Wood "	6–37	0–10	20–50	10–22	20–40
Bark 1928	12–27	34	10–22	16–36	0–25
Wood "	8–32	1–6	20–65	8–23	16–20

New growth starts with high concentration of amide and amino nitrogen in both bark and wood, which falls as the growth progresses, remains at minimum in autumn and winter, and rises when new buds begin to swell. Amide nitrogen in wood is much higher than the amino nitrogen in the period of active growth. Basic nitrogen is at its lowest in June, reaches its maximum some time before new growth starts, and then falls rapidly.

On hydrolysis humin nitrogen in the bark and rest nitrogen in the wood decrease considerably, causing a concurrent increase in amide, amino, and basic nitrogen.

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CHEMICAL EQUIVALENT BASE EXCHANGE REACTIONS IN PLANTS

W. T. McGeorge

The problem of determining the causes of the movement of mineral elements through membranous tissues within the plant has elicited considerable scientific inquiry. Moreover, the factors which govern the concentration of the several ions in plant sap and of those fixed by the plant tissues have received much attention. Any observations, therefore, which relate to these phases of plant physiology and nutrition are of interest.

During recent years much work has been done on the relationships existing between the soil solution and the exchange complex of the soil, the compounds which function in the stoichiometric exchange of bases. The soil compounds associated with base exchange reactions may be divided into organic and inorganic groups. The latter have been extensively investigated, but the former have received comparatively little attention. While the absorbing power of so-called humic bodies has been recognized for many years, comparatively little study has been devoted to quantitative values and to the nature of the absorbing compounds. At the time the exchange property of the clay fraction of the soil was demonstrated the same property was assumed for the organic fraction, but later some doubt arose as to the validity of this assumption. This problem has been rather intensively studied at the Arizona Agricultural Experiment Station (**3**) and it has been demonstrated beyond question that the organic fraction of the soil possesses a chemically equivalent exchange property, and that it is a linear function of the carbon content of the organic material.

Of the carbon compounds which compose the organic fraction of the soil the pentosans, lignins, proteins and cellulose are most abundant. Of these, lignin appeared most likely to possess base exchange properties and it was therefore chosen for study. While the chemical composition of lignin has not been definitely established, it is known to possess phenolic hydroxyl groups through which basic lignates may be formed. Lignin as isolated from a number of soils and from plant materials was studied and all samples were found to possess the property of exchanging one base for another in chemically equivalent proportions. On quantitatively determining the percentage of lignin and the replacement capacity of highly organic soils, a linear relationship between them was noted. The base-absorbing capacity of lignin varied with the source, and with the method used in preparing it from the mother substance. For example, lignin extracted from soils and plant material by alcoholic NaOH varied in exchange capacity from 38 to 178 M.E. (milliequivalents) per 100 grams, while that extracted by aqueous

NaOH varied from 327 to 420 M.E. per 100 grams. It was found that the maximum absorbing capacity of lignin depended upon complete hydrolysis of the complex. This is shown by the fact that the exchange capacities of lignins extracted with alcoholic NaOH were all increased to approximately 200 M.E. when hydrolyzed with HCl, while the capacities of the aqueous soluble lignins were increased to approximately 400 M.E. If we assume that lignin in raw plant material is in part combined with cellulose as lignocellulose this hydrolytic effect of HCl is easily explained.

Throughout the investigations (**3, 4**), which have been very briefly presented, the fact that lignin is a constituent of most plants was outstanding. This fact suggested that lignins, and possibly other organic compounds, notably the lipoids and pectins, may function in base exchange reactions within the plant. Absorption capacity would be limited by the combination with cellulose. Thus lignin may be said to exist in the plant in both active and inactive forms with the base exchange capacity varying accordingly.

Experimental

To investigate the base exchange phenomenon in plant material, alfalfa, one of the principal crops of the Southwest, was chosen for study. Five-gram samples of air dried, finely ground alfalfa were treated as follows:

1. Without any preliminary washing, duplicate samples were leached with neutral normal barium acetate and neutral normal ammonium acetate, 250-cc. volumes being used. Hydrogen was determined by electrometric titration of the barium acetate leachate and calcium, magnesium, potassium and sodium determined in the ammonium acetate leachate by gravimetric methods.

2. Duplicate samples were leached with 500 cc. of neutral 95 per cent. ethyl alcohol, after which they were leached with barium and ammonium acetates as in 1. The alcohol and acetate leachings were analyzed separately.

3. Duplicate samples were leached with 500 cc. of distilled water and then leached with barium and ammonium acetates as in 1. The water and acetate leachates were analyzed separately.

In each case where ammonium acetate was used, the samples were subsequently leached with alcohol to remove the excess ammonium acetate, and then the absorbed ammonium determined quantitatively. The data are given in table I.

That alfalfa possesses the property of exchanging its bases and hydrogen with other bases in chemically equivalent amounts is shown by a comparison of the total amount of ammonium absorbed and the sum of the displaced bases and hydrogen in column 3. In other experimental work similar data

TABLE I

TOTAL BASES, THE RELATIVE SOLUBILITIES IN ALCOHOL AND WATER, AND THE FIXED EXCHANGEABLE BASES

RESULTS ARE RECORDED IN MILLIEQUIVALENTS PER 100 GRAMS

	TOTAL BASES AND HYDROGEN	INSOLUBLE IN ALCOHOL BUT EXCHANGEABLE	INSOLUBLE IN WATER BUT EXCHANGEABLE
Exchange* capacity	44.1	41.6	39.6
Hydrogen	16.0	Trace	7.0
Calcium	78.0	63.0	27.8
Sodium	49.9	5.7	3.2
Magnesium	11.1	3.7	3.1
Potassium	30.2	3.9	0.15
		Dissolved by alcohol	Dissolved by water
Calcium		11.8	52.5
Sodium		51.1	52.3
Magnesium		9.9	9.7
Potassium		28.4	28.6
Total	185.2	177.5	184.35

* Calculated from the NH_4 absorbed from ammonium acetate.

have been obtained by determining the saturation capacity of alfalfa for barium and calcium ions. Column 1 of table I shows the total amount of bases and hydrogen present both as soluble and as fixed forms. In this case the soluble cations are approximately three times the fixed ions on a milliequivalent basis.

Alcohol was used as a leaching solvent to determine whether or not the soluble unfixed cations could be removed preparatory to determining the fixed cations. It will be noted that the calcium is difficultly soluble in alcohol, while the sodium, potassium and magnesium are largely removed by this solvent. Alcohol, therefore, fails to qualify as a preliminary leaching liquid in the determination of exchangeable bases.

Column 3 closely represents the ratio of bases in fixed and soluble forms, although there is some evidence that a part of the fixed potassium is displaced by soluble calcium during the leaching operation.

In addition to demonstrating an equivalent exchange property of bases, the data show that calcium is the predominant fixed base. The essential need of this high calcium absorption is manifested in the property of the plant material when saturated with calcium and with an univalent base,

such as sodium or potassium. Leaching was very rapid with the calcium saturated alfalfa, but when saturated with sodium or potassium the plant tissues became swollen and dispersed, and leaching velocity was greatly reduced. Tissue permeability is therefore a function of calcium saturation and will be reduced if too great a displacement of calcium by sodium or potassium takes place.

Common ion effect

In base exchange reactions the energy of absorption of the exchange complex is of the order Ca > Mg > K > Na and is therefore, for the bases which have been discussed, greatest for calcium. On the other hand, absorption is also influenced by the concentration of the salt of a common base and other electrolytes in the control solution. The property of ionization of basic lignates and the effect of common ions on this property has been demonstrated (**4**). Similar experiments with alfalfa were also conducted.

Six 2-gram samples of alfalfa were leached with neutral 0.5 normal calcium acetate, after which the excess salt was removed by leaching with water. These calcium saturated samples were then placed in 500-cc. volumes of varying concentrations of KCl solution, shaken occasionally for 4 days and the displacing effect of the K ions ascertained by determining the Ca in solution. The results are given in table II.

TABLE II

REPLACEMENT OF CA FROM CA-SATURATED ALFALFA IN CONTACT WITH VARYING CONCENTRATIONS OF KCL

2 GRAMS ALFALFA 500 CC. OF SOLUTION

EXPERIMENT NO.	KCL PER 500 CC.	FIXED CA IN 2 GM. ALFALFA	CA DISPLACED BY KCL
	gm.	*gm.*	*gm.*
1	None	0.0160	0.0016
2	0.5	"	0.0032
3	1.0	"	0.0036
4	2.5	"	0.0041
5	5.0	"	0.0044
6	10.0	"	0.0045

A similar experiment was conducted in which the potassium-saturated alfalfa was prepared by leaching with a solution of potassium acetate and finally with 95 per cent. alcohol to remove excess of salt. These samples were then placed in contact with 500-cc. volumes of varying concentrations of $CaCl_2$ solution, shaken occasionally for 4 days and displaced potassium ascertained by determining the calcium left in solution. The results are given in table III.

These data clearly illustrate the greater energy of absorption for calcium. With a solution of 10 gm. KCl per 500 cc. in contact with 2 gm. calcium-

TABLE III

DISPLACEMENT OF K FROM K-SATURATED ALFALFA IN CONTACT WITH $CaCl_2$ SOLUTIONS
2 GRAMS ALFALFA PER 500 CC. SOLUTION

EXPERIMENT NO.	$CaCl_2$ PER 500 CC.	CA ABSORBED BY ALFALFA	ABSORBING CAPACITY OF 2 GM. ALFALFA IN GM. CA
	gm.	*gm.*	*gm.*
1	0.1	0.00780	0.0169
2	0.5	0.00997	0.0169
3	1.0	0.01490	0.0169

saturated alfalfa, only one-fourth the calcium was displaced. On the other hand, when the potassium-saturated alfalfa was placed in contact with solutions of $CaCl_2$ the displacement of K was almost complete in the presence of 1 gm. $CaCl_2$ per 500 cc. These data lend confirmation to those in table I and illustrate why the absorption capacity of the plant is largely saturated with calcium. Water permeability and turgidity of the tissues apparently demand a near saturation with calcium.

The reactions described, the data presented and the observations which have been made, may be used to explain a number of experimental observations to be found in the literature where mention is often made of the abnormal changes in permeability of plant cells subjected to single salt cultures. According to OSTERHOUT (**5**) the antagonistic effect of salts is due to the presence in the cell of a compound regulating permeability. HANSTEEN-CRANNER (**2**) suggests that lipoid and pectic materials govern permeability of cell walls. Alkalis act as dispersants and calcium as a coagulant. PRIESTLEY (**6**) found a similar property for pectic acids, namely, the formation of gelatinous soluble salts with sodium and potassium, and of insoluble flocculent salts with calcium. These statements agree with our own observations on the property of the alkali- and alkali earth-saturated alfalfa. GENAUD (**1**) has recently demonstrated a rapid ionic interchange between the cellular membrane of yeast cells and the surrounding fluid. The cationic interchange usually involves equimolecular quantities. He also found that bivalent ions penetrate more easily than univalents. The cell membrane and cell vacuole behave like insoluble organic acids, exchanging their cations for those of the environing medium until an equilibrium, due to mass action, is obtained.

It is also of interest to note that STILES (**8**) greatly emphasizes the failure of osmotic views to afford a complete explanation of the cell reaction to its dissolved substances. He observed (**7**) that when storage tissue is immersed in a solution of a single salt, the ions of the salt are not absorbed in equivalent amounts. The balance of the ionic charges in the external solution is maintained by the diffusion of ions from the tissue. He

also mentions that salts may combine chemically with the cell constituents. STILES also quotes similar work by REDFREN (7). In the latter's experiments it was found that on immersing tissues in a solution of $CaCl_2$ the calcium was absorbed in excess of the Cl and that Mg and K ions had diffused out of the tissue, being replaced in the tissue by calcium.

These few references serve to show that there is every reason to suppose that certain tissue constituents play a part in the interchange of substances between plant membranes. It is shown by our experiments that absorbed bases may be displaced by other bases or by hydrogen, and that this interchange may play a part in ionic movement.

Summary

Air dried plant tissues of alfalfa show a definite property of absorbing bases or hydrogen and exchanging them for other bases in chemically equivalent proportions. It is suggested that living tissues may exhibit this same property and that it may be related to the phenomenon of the permeability of plant tissues and cationic concentration within the plant system. The diffusion of bases through the plant tissues may be aided by an actual combination of the base with a relatively insoluble anion, such as lignin for example, to form a definite chemical combination, or to form an absorption complex with other electronegative colloids which are tissue constituents.

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RAPIDITY OF RESPONSE OF SPINACH TO CHANGE IN PHOTOPERIOD

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When plants are shifted abruptly from existing photoperiodic conditions to a day-length favorable for flowering, changes in the metabolism of the plant result ultimately in flower-bud formation. The change thus initiated should be easier to demonstrate than the gradual shift from a vegetative to a flowering stage which accompanies the natural variation in length of day. These experiments were conducted to see how quickly a change could be noted in the plant after a change in photoperiod.

GARNER, BACON and ALLARD (**1**) found a sharp decline in the hydrogen-ion concentration of the sap of the region of the growing points of a number of species of plants. This occurred three to five days after the plants were transferred to a short day. They believed this to mark definitely the actual transition from the vegetative to the flowering stage.

The catalase activity of the tissues of the growing point has been shown (**3, 4**) to be closely associated with the vegetative or reproductive condition of the bud. As the seed-stalk increased in height the catalase activity decreased. Unpublished data obtained in connection with earlier studies (**4**) indicated that there was a considerable increase in catalase activity of cosmos buds the morning following the sudden change from a long to a short day, although some days later the catalase activity decreased. Further, it seemed that this increase in catalase activity could be noted within a few hours of the change.

A vegetative spinach plant may have a stem height of 5 to 7 mm. as measured from the junction of the root and stem. Thus in data previously presented (**3**) which showed a correlation between the increase in height of seed-stalk and the decrease in catalase activity of the apical bud, there were 43 of the 143 individuals which had a seed-stalk height of less than 10 mm. This stage of development was accompanied by a high catalase activity of the bud. It was impossible to say whether or not this level of catalase activity was higher than it was before the plants were exposed to the longer photoperiod. Seed-stalk development had not proceeded far enough for elongation to be visible. Nor had the bud reached the stage which would be accompanied by a decline in the catalase activity.

Methods

Stock seed of two varieties of spinach developed by the Virginia Truck Experiment Station was planted on September 24, 1930, and January 30,

1931, in a ground bed of the greenhouse. Six 16-foot rows of each variety, spaced 12 inches apart, were planted. Virginia Savoy is a variety which will produce a seed-stalk quickly under favorable conditions. Old Dominion is much slower in changing from the vegetative to the reproductive stage.

The temperature of the house was kept within the range of 55 to 60° F. For a week preceding the start of experiment 1, the spinach had been covered with black sateen at 4:30 P. M. Beginning on the afternoon of December 15th, a section across the eight rows was given a 5.5-hour increase in photoperiod. Four 100 watt bulbs were suspended 30 inches above the plants. The remainder of the bed was covered with a double thickness of black sateen while the lights were in operation. This cloth was moved along and the lights advanced as successive sections of the bed were exposed to the long photoperiod.

In the case of the second planting the whole bed was covered with black sateen each afternoon at such time as to keep the plants under short-day conditions. This cloth was removed about 9 o'clock at night.

To produce the change in photoperiod a strip across the bed was left exposed to the normal daylight, reinforced with artificial light as with the first planting. A screen was provided to prevent the direct sunlight from striking the cloth laid on the plants. Thus heating of the air below the cloth was avoided. The temperature of the house was higher than 60° F. on some of the warm sunny days during April.

Plants were taken from the bed at the times indicated in table I, placed in baskets covered with a damp cloth and removed to the laboratory. The leaves were stripped off until just the small center ones remained. This bud was cut where it joined the stem. One-third as much tissue was used in this experiment as in that with spinach previously reported (**3**). Thus the quantity of fresh tissue varied from 20 to 25 mg. It was macerated with an equal weight of precipitated chalk and 0.1 cc. of distilled water. Sufficient water was added to equal 50 times the weight of the bud tissues. Seven-tenths of a cubic centimeter of the catalase preparation was placed directly in one arm of the reaction tube. Cotton was used to stopper the tubes which were kept in an ice box at 38° F. until 9 o'clock the following morning. The cotton was removed and the contents of the tubes exposed to the air for ten minutes before the catalase activity was determined. One cubic centimeter of neutralized hydrogen peroxide (Dioxogen) was placed in the other arm of the tube. The catalase activity was determined for twelve samples at a time and recorded mechanically by the use of HEINICKE'S apparatus (**2**). The temperature of the bath was kept at 20° C., and the time of agitation was 180 seconds.

TABLE I

RAPIDITY OF RESPONSE OF SPINACH TO CHANGES IN LENGTH OF DAY AS INDICATED BY THE CATALASE ACTIVITY

EXPERIMENT NUMBER	SHIFTED TO LONG DAY BEGINNING		TIME OF SAMPLING		AVERAGE NUMBER OF SECONDS FOR EVOLUTION OF 1 CC. OF OXYGEN		INCREASE IN CATALASE ACTIVITY IN LONG-DAY PLANTS
					SHORT-DAY PLANT	LONG-DAY PLANT	
1	Dec. 15	4:30 P. M.	Dec. 17	9:00 A. M.	A 20.0 ± 0.45*	10.7 ± 0.13*	9.3 ± 0.48
					B 47.1 ± 0.96	35.4 ± 1.22	11.7 ± 1.55
2	Dec. 16	4:30 P. M.	Dec. 18	9:00 A. M.	A 29.6 ± 0.85	25.2 ± 0.71	4.4 ± 1.11
					B 29.4 ± 0.76	26.4 ± 0.39	3.0 ± 0.85
3	Dec. 18	4:30 P. M.	Dec. 19	9:00 A. M.	A 29.1 ± 0.99*	14.7 ± 0.15*	14.4 ± 1.00
					B 28.3 ± 0.63	25.0 ± 0.44	3.3 ± 0.77
4	Dec. 21	4:30 P. M.	Dec. 22	9:00 A. M.	A 41.5 ± 1.06	23.4 ± 0.24	18.1 ± 1.09
					B 52.1 ± 1.53	22.9 ± 0.53	29.2 ± 1.62
5	Dec. 23	4:30 P. M.	Dec. 23	7:00 P. M.	A 25.3 ± 0.68	22.4 ± 0.72	2.9 ± 0.98
					B 27.1 ± 0.64	36.1 ± 1.08	−9.0 ± 1.26
6	April 28	3:00 P. M.	April 28	5:30 P. M.	A 112.7 ± 3.45	67.7 ± 2.68	44.9 ± 4.37
					B 139.5 ± 2.29	118.2 ± 3.91	21.3 ± 4.54
7	April 30	3:00 P. M.	April 30	5:30 P. M.	A 64.8 ± 1.92	53.7 ± 0.90	11.1 ± 2.12
					B 59.4 ± 1.73	54.2 ± 1.64	5.2 ± 2.38
8	May 1	3:00 P. M.	May 1	5:30 P. M.	B 57.1 ± 1.18	57.7 ± 0.83	−0.6 ± 1.44
9	May 4	3:00 P. M.	May 4	5:30 P. M.	B 66.4 ± 1.59	58.9 ± 1.23	7.5 ± 2.01

* Seconds per half cubic centimeter instead of one cubic centimeter.

A = Virginia Savoy, B = Old Dominion.

Discussion of results

The results of these experiments are summarized in table I. Not all of the plants were equal in size, although selected as nearly as possible for this characteristic. Therefore there is some variation in their catalase activity.

The catalase activity was measured for 10 to 18 plants from the short- and the long-day lots of each variety at each time of sampling. Of the 357 determinations there were only 19 in which as many as 3 of the 12 cubic centimeters of oxygen available in the Dioxogen were released. There were 88 which gave as much as 2 cubic centimeters. In most cases the time for the evolution of 1 cubic centimeter of oxygen appeared to be the best basis of comparison, but in the two cases indicated in the table, it was necessary to use the time for one-half cubic centimeter.

The data in table I indicate that there is an increase in the catalase activity of the apical bud of spinach following a change from short to long photoperiod. This increase is evident 16.5 and 40.5 hours after the change. The change is more marked in Viriginia Savoy than in Old Dominion, except in experiment 4.

It will be noted that the catalase activity of the short-day plants has fluctuated from one experiment to the next, due probably to the application of water to the bed, as this was supplied as the soil dried. Thus, more was needed on sunny days, and the daily application of water could not be constant. The two days preceding the start of experiment 6 were cloudy and water was applied the morning of April 28. This appears to be the reason for the difference in the level of catalase activity as compared to that in experiments 7, 8 and 9 where water was applied 30 hours before the start of each experiment. The plants had almost full sunlight the day before and the day of each of these last three experiments.

Experiments 5 to 9 were designed to check the earlier observation with cosmos which suggested that the sudden change in day length might influence the catalase activity within a few hours.

In experiment 5 the increase in catalase activity in Virginia Savoy is not quite great enough to give odds of significance, but indicates that there had been a disturbance of the metabolism. The decrease in Old Dominion in experiment 5 can be explained by the condensation of water on the bud leaves as these plants stood in a moist atmosphere at a temperature slightly above freezing before they were macerated. Although an attempt was made to dry them by touching the surface with dry cheesecloth, enough water remained to cause error in weighing and consequently in the degree of dilution.

Experiments 6 to 9 show that the Virginia Savoy variety has shown a greater increase in activity 2.5 hours after the sudden change in photoperiod,

than has Old Dominion. Only in experiments 6 and 9 is the increase in catalase activity of Old Dominion significant.

Plants which had been exposed to the long photoperiod from December 15 and December 24 were measured for seed-stalk height on January 5, 1931. These data are presented in table II and show that it took 21 days for the Old Dominion to reach the height of seed-stalk attained by Virginia Savoy in 12 days under the temperature conditions prevailing. At the end of 12 days, Old Dominion had scarcely started to elongate, as the base of the plant itself may be 5 to 7 mm. high. These figures indicate why a more pronounced and more rapid change in catalase activity might be expected in Virginia Savoy.

TABLE II

AVERAGE HEIGHT OF SEED-STALK OF VIRGINIA SAVOY AND OLD DOMINION SPINACH AFTER CERTAIN PERIODS OF EXPOSURE TO A LONG PHOTOPERIOD

VARIETY	NUMBER OF DAYS OF EXPOSURE TO LONG PHOTOPERIOD	
	12 DAYS HEIGHT OF SEED-STALK	21 DAYS HEIGHT OF SEED-STALK
Virginia Savoy	38.75 ± 2.397 mm.	90.34 ± 6.411 mm.
Old Dominion	10.00 ± 0.675 mm.	39.58 ± 1.569 mm.

The increase in catalase activity of the apical bud soon after a change to a photoperiod favorable for flowering is followed later by a decrease. The hydrogen-ion concentration of the apical tissue has been shown to decrease and then increase under similar conditions (**1**). However, the change in catalase seems to occur more quickly. The two may be independent, but simply manifestations of the change in metabolism occurring in the shift from a vegetative to a reproductive type of growth.

Summary

Determinations of the catalase activity of 20 to 25 mg. of fresh apical bud tissue of spinach plants, taken 2.5, 16.5 and 40.5 hours after a change to a longer photoperiod, show that the plants have responded to the stimulus by an increase in the catalase activity. This indicates that spinach has responded rapidly to the change in day length.

The Virginia Savoy variety which produces seed-stalks more quickly than the Old Dominion variety under a favorable photoperiod has shown a greater change in the catalase activity.

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MODE OF OCCURRENCE OF CAOUTCHOUC IN THE GUAYULE, *PARTHENIUM ARGENTATUM* GRAY, AND ITS FUNCTION

FRANCIS E. LLOYD

(WITH ONE PLATE)

The account which I published in 1911 (**9**) of the mode of occurrence of caoutchouc in guayule, *Parthenium argentatum* Gray, is incorrect. I was led into error by inadequacy of both method and point of view. The purpose of the present paper is to set the matter right, so far as I now understand it.

In the guayule, as in some other rubber-bearing plants, the rubber occurs in the parenchyma cells and is thus segregated. In contrast with this condition is that in the so-called latex-bearing rubber plants, such as *Hevea, Manihot, Ficus, Euphorbia, Scorzonera, Chondrilla,* etc., in which the rubber is a constituent, more or less prominent according to the species, of a white or colored milky fluid, which is stored in tubes from which, when opened, the fluid flows more or less freely. The fluid in question is always a complex emulsion of rubber[1] and other substances (resins, fats) as the internal phase, with water containing proteins, salts, sugars, etc., in solution as the external phase. The size of the suspensoids differs greatly, and is from 2 microns and less in *Hevea* and *Kickxia* to 60 microns or more in *Musa.* This general statement may now be extended to the guayule for, as will be shown, the rubber associated with other substances occurs in the same manner. The fluid here is equally a latex[2] confined to individual cells. In *Hevea,* etc., it occurs originally in individual cells which break down in series to form tubes, or, as in the seed, in cells which tend to form inarticu-

[1] Latex is, of course, not always rubber-bearing (see MOLISCH, **14**; LLOYD, **12**).

[2] The word "latex," while meaning merely a "fluid," has come to be used more specifically for milky rubber-bearing fluids occurring in tubular structures (Oxford Dictionary). It is, however, impossible to adhere to this usage rigorously. For example, the milky exudate from cut surfaces of *Musa* is always spoken of as latex in the more restricted sense; as a matter of fact, this latex is merely the sap (latex in the original sense) of large barrel-shaped cells placed in series like a string of beads, each cell separated from its neighbor by a thin circular wall (double, of course). On cutting fresh living tissue, the internal pressures break down the walls, hence the exudate. If the part cut is fully wilted or has otherwise lost its turgor (as when a banana fruit has been "chilled" (LLOYD, **11**) no exudation takes place. The word "latex" is here obviously misapplied unless used in its primary sense. I should prefer, therefore, to adhere to the primary meaning, coupled with qualifying terms, as parenchyma latex (*Parthenium, Musa*), vascular latex (*Euphorbia, Hevea*). It is well understood the latex vessels of *Hevea* arise by the resorption of the contiguous walls of seriate members. "Sap" could be restricted to the fluid of wood vessels.

late tubes (PARKIN, **15**), such as those which occur in many other plants, *e. g., Argemone* (TRECUL, **18**).

The evidence as to the nature of the parenchyma latex of guayule is very simple. If a living plant stem be cut across and the cut surface be dipped into a drop of water on a glass slide and examined at once, the water will be observed to have become milky. Placed under the microscope, it is seen to be a rich suspension of minute particles in lively Brownian movement. If now a section be cut, thick enough to afford uncut cells but thin enough so that uncut cells do not overlie each other, and examined microscopically, the contents of the cells appear as a densely milky fluid, more especially if observed in reflected light with the dark field condenser. In transmitted light, the contents of the cells appear cloudy and more or less obscure, according to their density. At the same time, one may observe that the minute particles or suspensoids in the fluid in each cell are in Brownian movement, the observation of which is most easily made in cells which are not too fully packed with the suspensoids, for obvious reasons. A series of preparations made from a shrub containing poorly to richly loaded cells will afford a variety of pictures, from cells containing only a few minute suspensoids to those containing a very dense suspension. Such preparations, if photographed with a time exposure, give a curious result pictorially, which of course could be expected, namely, cells appearing as if filled with an ill-defined cloud (plate II, figs. 1, 3). It is only when a rapid exposure is used that the individual particles may be resolved, for which purpose not too well filled cells are best (plate II, fig. 5). An instantaneous photograph of diluted cell fluid affords a picture in which one may obtain an impression of the range of size of the particles, which extends from two microns to those of so minute a size as to be just within the limits of resolvability (plate II, fig. 8). The particles are all spherical, as in the latex of *Ficus*, and not pear-shaped, as are many of the suspensoids of *Hevea*. It is not, of course, to be supposed that all the particles are caoutchouc in guayule any more than in other rubber-bearing plants. But that a part of them, indeed the greater part, is caoutchouc may be proved by the use of suitable solvents for other possible components, such as resins, proteins, oils. Sections treated with such solvents for sufficient periods of time still reveal suspensoids remaining undissolved, provided only that a solvent for caoutchouc has not been used. If, however, for such tests cells are used which contain a great deal of rubber, this will have become coagulated. If, on the other hand, cells with a small amount are used, the particles may remain discrete though they usually become more or less adherent to various surfaces present—the mere result of space relations.

If now coagulating agents are used at once, the coagulation of the latex within the individual cells proceeds and there results within each cell sev-

eral coagula if the amount of rubber is small—that is to say, flocculation takes place (plate II, figs. 4, 6); or, if there is enough rubber, a single clot is formed (plate II, figs. 2, 7). This, as is well shown in the figures in my monograph (1911), is not homogeneous internally, but contains inclusions of fluid—are vacuolated, so to speak—and particles of non-rubber constituents (plate II, fig. 7). The change is irreversible.

The above described behavior in the guayule is of interest both theoretically and practically. From the point of view of physiology it was difficult to regard the caoutchouc, if laid down in the parenchyma cells as solid masses, as a food or other reserve. The question of the function of latex, or, to particularize, of any or all of the constituents of the latex, has puzzled every one who has taken any theoretical interest in rubber plants, for the reason, if for no other, that if any or all of these constituents are physiologically useful, there might be expected some variation in the composition of the latex at one time or another in the cycle of growth, let us say, and thus during the year the crop of latex would be uneven in quality or quantity or both. While in the case of *Hevea* there seem to be minor differences in composition of the latex as between tree and tree, or more obviously between race and race, the differences are slight and appear to indicate nothing in regard to physiological significance. There is also the now known effect of enrichment of the soil by sodium nitrate, etc., in increasing the yield of latex (GRANTHAM, **6**), but it can be inferred that this effect is general, affecting many if not all features related to growth. Possibly the number of stone-cells in the cortex would be affected, but this feature is so different quantitatively in various races of *Hevea* that it would require hardihood to claim a physiological function for the stone-cell. Some would perhaps argue for a protective effect, but there is no evidence that this is the case: just as a similar effect has been argued for latex (DE VRIES, **19**) in the sealing of wounds, without any real evidence that *Hevea* is less subject to the ills of the flesh than other non-latex producing plants. However, it may be suggested that the coagulative capacity of rubber and other latices serve for the prompt closure of wounds so that the internal pressures, which are high (MOLISCH, **14**) may be restored and preserved.

As to the experimental evidence in support of the idea that latex is in part at least a fund for energy supply, there is the evidence adduced by FAIVRE (**3, 4, 5**) for *Tragopogon porrifolius, Morus alba,* and a *Ficus.* By varying conditions (including absence of carbon dioxide) he observed impoverishment of the latex in the above forms, and inferred their nutritive value. His views were favorably received by DE VRIES (*l. c.,* **19**). CH. BERNARD (**1**) also did experiments with *Euphorbia thymifolia* and *E. splendens* exposed to CO_2-free air and observed the corrosion of the starch grains; and what happens to one component of the latex may happen to an-

other. PFEFFER (**16**), however, asserts that among other substances India rubber is "incapable of further metabolism," which remark, whether eventually shown to be true or not, serves to indicate the crux of the problem for us at the moment. The only additional experimental evidence that is available is contained in a note by D. SPENCE (**17**), who states that in a young *Ficus elastica* "grown in an atmosphere and soil free of carbon dioxide, gradually drew on their milk which became nothing more than water after a few weeks' time." This experiment, supporting the conclusion of FAIVRE (**3**), indicates that in the absence of a raw material for the elaboration of a hydrocarbon, here caoutchouc, the supply already present may be metabolized. The presence of suitable enzymes, shown in the first instance by SPENCE (**17**) for *Hevea* and *Funtumia,* coupled with the great surface area of the suspensoids, lends support to the idea. The very condition in which the latex occurs, namely, in a vascular system, or system of tubes, their very wide distribution in the plant kingdom, and the evident nutritive values of many of the latex constituents predispose only to the view that, in general, latex is not merely a depository of wastes.

In considering the guayule and other plants which have and hold their caoutchouc in like manner, namely, in the individual cells of the parenchyma, the predisposition is to compare this condition to analogous ones in which known metabolically useful materials are accumulated, *e. g.,* starch in the potato tuber. In the latter it is easy to demonstrate experimentally that the starch is actually and measurably used. Now that we know that the caoutchouc occurs as a colloidal suspension, precisely as in the latex of *Hevea,* etc., we have no difficulty in accepting the possibility of its attack by enzymes: what seemed difficult, holding the older and incorrect idea that the caoutchouc is disposed in solid masses, a single one in each cell, is now easy when the surface relations inherent in a colloidal suspension are realized (MOLISCH, **14**, p. 80). On general principles there is no particular difficulty in expecting even so refractory a substance as rubber to be acted upon readily if the proper agencies (enzymes) are present. This would be merely the reversal of the anabolic process. This argument has been used by MOLISCH (*l. c.*) in regard to the particulate condition of the other substances in latex.

That, however, the hydrocarbon in question is, in the case of the guayule, actually a reserve substance and is as actually made use of in metabolism requires proof, which has not yet appeared. We know that during periods of rapid growth very little caoutchouc is formed, this happening after the cessation of growth during the drought period following the rain which made growth possible. The amount of rubber which can occur in maximum quantity (*ca.* 20 per cent.) depends not alone on the particular race of guayule (MCCALLUM, **13**), but also on the rapidity of growth and the length

of the drought following cessation of growth. At all events, in the semi-arid deserts in which it occurs, the guayule accumulates rubber until some maximum is reached and the amount then appears to remain constant until a new growth period sets in. The addition of new tissues during the growth period makes it very difficult to determine whether meanwhile the caoutchouc is being drawn upon as a source of energy. The solution of the problem is one of the most interesting in the field of plant metabolism and it is to be hoped that those favorably placed and interested will find it possible to find the solution.

The view that the caoutchouc in the guayule confers on the plant some ability to withstand drought has been advanced from time to time, but no convincing evidence has accrued. That the plant wilts at once on being pulled suggests a negative answer. On the other hand, plants so situated that they must withstand a drought period are subjected to a very gradually effective drying out which might affect the leaves without affecting the stems, while these may have a superior drought resistance conferred on them by the presence of the caoutchouc. The fact that it requires a considerable period of time after the shrub has been "pulled" before it "cures" so that it can be milled might be taken as an indication that during some period—probably a short one—the parenchyma cells retain their vitality as indicated by the dispersed condition of the contents of the cells.

On the other hand, closely related species, such as the mariola (*Parthenium lyratum*), which appear to be equally able to withstand drought, if not more so, have very little caoutchouc; but here we impinge on one of the very difficult problems in the field of adaptation, the question of how far the same ends are attained by different means.

An additional suggestion is prompted by the recent work of WALTER (**20**), who reports that drought resistance of leaves (the stems were not investigated) "varies directly with the maximum osmotic value and with the osmotic inertia of the plant," namely, that the accumulation of caoutchouc and water-soluble substances such as oils and some resins may increase the osmotic value of the sap merely by occupying space, thus by indirect means gradually changing this from a lower value during the continuation of drought.

The practical interest attaching to the matter of the condition of the caoutchouc is well understood by those who during the past twenty-eight years have been concerned in the extraction of rubber from the guayule shrub. It has long been known in northern Mexico that by merely chewing the stems, rubber ("hule") could be obtained, and when communal methods were used a sufficient amount could be obtained to make balls for playing games. Rubber was well known to the prehistoric peoples of Mexico, who made small images, utensils and balls, etc. It was obtained

by coagulating the latex of *Castilloa;* and it is not unlikely that such objects found their way as far north as southern Arizona (LLOYD, **10**). It is, however, pretty certain that in northern Mexico the rubber of guayule was also used (LLOYD, **9**). At any rate, the method of obtaining small quantities by mastication is and has been general knowledge among the peons of northern Mexico for a long time, certainly previous to the beginnings of the manufacture of guayule rubber just previous to 1903. It was in this year that Mr. WILLIAM A. LAWRENCE discovered that this substance could be extracted from the shrub by a strictly mechanical process of comminution and flotation in water, by which the floating rubber could be separated from the bagasse (CARNAHAN, **2**). For many years thereafter, however, there were measurable and even serious losses due to various imperfections in the methods of manufacture, following from imperfect knowledge of what constituted "curing" the shrub, which we now know to be the time and conditions for bringing all the suspensoids into the coagulated condition.[3] With the fact before us that the rubber in the guayule exists as a colloidal suspension, experimentation can proceed with a perfectly definite criterion before the investigator. It is one so simple, moreover, that it is a matter of surprise that it was not long ago discovered, namely, the method indicated above. One needs but to make a cut across the tissues and touch the cut surface to a drop of clean water on the piece of glass. If the rubber exists as suspensoids, they will flow out and render the water milky. Slight turbidity may be determined by putting a few slices of tissue in a small volume of water and then determining the turbidity by means of a strong beam of light (TYNDALL effect). For the purpose of checking this method of treatment, the dried material, after allowing a drop to dry on the glass, may be treated with acetone to remove the resins, and the adhering caoutchouc then examined, when, of course, staining may be used.

At this point I wish to acknowledge the courtesies extended by Mr. GEORGE H. CARNAHAN, President of the Inter-Continental Rubber Company, who made it possible to re-examine living guayule, both at Salinas, California, and at Torreon, Mexico, in 1926 and 1927. It is with his concurrence that the above notes are published.

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[3] Since such plants as *Scorzonera* and *Chondrilla,* certain species of which are being exploited by the Russians, are vascular latex plants, and are hardly susceptible of being tapped, the latex in them must be allowed or caused to coagulate, before extraction, thus following the method of "pilonage" used in Africa for the extraction of the rubber from *Landolphia heudelotii* (JUMELLE, 7); that is, by a method fundamentally similar to that used for guayule.

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Explanation of Plate II

Fig. 1. Section of the living cortex, including a portion of a resin canal, of *Parthenium argentatum* Gray. The cells appear as if filled each with a dense cloud. This is the result of a time exposure on many suspensoids in Brownian movements. Compare with figure 5.

Fig. 2. The same section after brief treatment with weak acetic acid. The latex within the cells has now been coagulated in nearly solid masses, almost filling them.

Fig. 3. Living cells from the pith, time exposure. The cloudy appearance of the interior of the cells is owing to the movement of the suspensoids, as in figure 1.

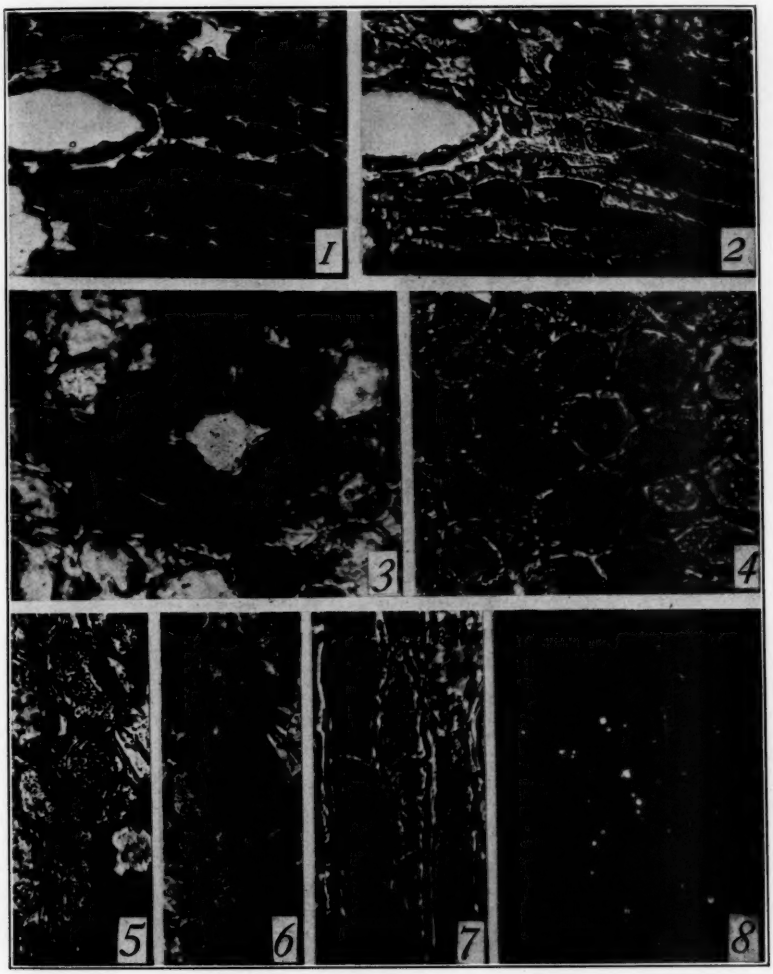
Fig. 4. The same section as in figure 3, after treatment with acetic acid. The amount of material in the form of suspensoids is not great enough to make a solid mass, but is merely flocculated. Contraction follows later.

Fig. 5. Instantaneous photograph of three undamaged cells of the cortex, showing the suspensoids of caoutchouc (for the most part). A time exposure would have yielded a result such as in figures 1 or 3.

Fig. 6. The same after treatment with acetic acid.

Fig. 7. A larger picture of such cells as are seen in figure 2 showing that after coagulation the coagula are not homogeneous.

Fig. 8. Dark-field photograph of the parenchyma latex diluted with water.



LLOYD: CAOUTCHOUC IN GUAYULE

OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION DURING THE GROWTH OF YEAST

OSCAR W. RICHARDS AND FLORENCE W. HAYNES[1]

(WITH THREE FIGURES)

In the present experiments the oxygen consumption and carbon dioxide production of a pure strain of yeast have been investigated throughout the first and second cycles of its growth in a synthetic nutrient solution. Few measurements of the respiration of yeast in the literature are comparable to these observations, since other investigators have used different kinds of yeast under various experimental conditions which have not always been stated clearly. The measurements have usually been made only for short intervals of time. COPPING (**2**) has published curves for the oxygen consumption, carbon dioxide production and cell count of *Saccharomyces cerevisiae* on different media for a period of about four days but readings were made only at daily intervals and no attempt was made to correlate the gaseous exchange of the yeast with its growth.

For these experiments a pure strain of *Saccharomyces cerevisiae* Hansen previously obtained from a single cell (RICHARDS, **5**)[2] was grown in WILLIAM'S medium.[3] The seedings were made with 0.10 cc. of a suspension from yeast cultures of about the same age and the technique of measuring the multiplication of the yeast was that of RICHARDS (**6**).

The oxygen consumption and carbon dioxide production of the yeast were determined with differential microrespirometers of the type devised by THUNBERG (**9**) and more recently described by FENN (**3**). For the measurements of oxygen consumption 0.5 cc. portions of 20 per cent. sodium hydroxide were introduced into small cups in the two chambers of the respirometer to absorb carbon dioxide. Into one chamber was placed 1 cc. of medium containing yeast and into the other enough water to make the air spaces equal. As oxygen was consumed the index drop in a horizontal capillary connecting the two chambers was drawn toward the chamber containing the yeast. The oxygen consumption was obtained by multiplying the movement of the drop in centimeters per minute by the volume of the capillary in cubic centimeters per centimeter. Twice this value was used

[1] The measurements of respiration were made by Miss HAYNES and the growth counts by RICHARDS.

[2] Isolated from a cell of yeast no. 2335 furnished to RICHARDS through the courtesy of Dr. F. W. TANNER.

[3] Sucrose 20 gm.; $(NH_4)_2SO_4$, 3 gm.; KH_2PO_4, 2 gm.; asparagine, 1.5 gm.; $CaCl_2$, 0.25 gm.; $MgSO_4$, 0.25 gm.; distilled H_2O, 1,000 cc. All chemicals were of Merck's Blue Label reagent quality.

since the second, compensating chamber reduces the movement of the drop by one-half. When measuring the carbon dioxide production the sodium hydroxide was omitted, so that the value for the CO_2/O_2 was obtained directly. The addition of the amount of oxygen (O_2) consumed to this value gives approximately the carbon dioxide (CO_2) produced.

The apparatus was suspended in a water-bath in which the temperature was maintained between 28.2° and 28.3° C. About every 12 hours cell counts were made on control tubes in the same bath. Carbon dioxide was not removed from these tubes with sodium hydroxide because previous experiments[4] had shown that such a removal of carbon dioxide did not significantly influence the growth of the yeast. The cotton stoppers were not tight enough to prevent the escape of the gas. The growth curve is an average curve. As the yields in the several respirometers varied slightly owing to the technical difficulty of getting exactly the same seeding in each, the readings were brought together at the end of the growth period (*cf.* RICHARDS, **6**).

The experiments lasted approximately 130 hours and readings were taken every 2 to 5 or more hours. The oxygen consumption was averaged over the intervening period. Because of the large amounts of carbon dioxide produced during the fermentative phase of the yeast growth, measurements of the rate of carbon dioxide produced were made every few hours for short

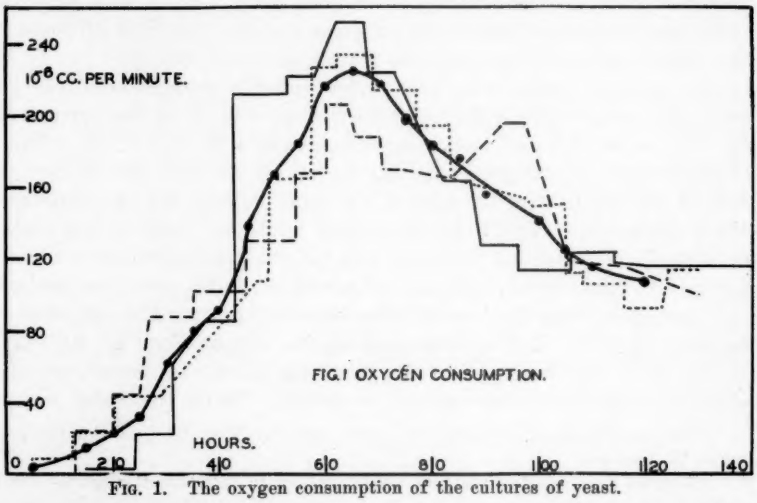


FIG. 1. The oxygen consumption of the cultures of yeast.

[4] The slight increase in the growth of the yeast when the CO_2 was removed by suspending a short vial containing NaOH above the culture medium was less than the variation of the crop in the respirometers.

intervals of time. As the chambers were frequently opened to the air in order to return the index drop to its initial position, there was probably no oxygen depletion. After these manipulations time was allowed for the equilibrium to become established before making measurements.

The oxygen consumption is plotted in fig. 1 from a series of three experiments. The determinations of the individual experiments (averaged over 3–13-hour periods) are plotted as horizontal lines. These are joined by vertical lines to show the parts of a single experiment. The continuous curved line is the average curve obtained by drawing the curve through average points taken at 5-hour intervals. The rate of oxygen consumption, shown by this average curve, increases until about 65 hours after seeding and then decreases until it reaches an equilibrium at about 130 hours.

The rate of growth, fig. 2, is greatest at about 30 hours, returns to zero at the equilibrium between the first and second cycles of growth (65–70)

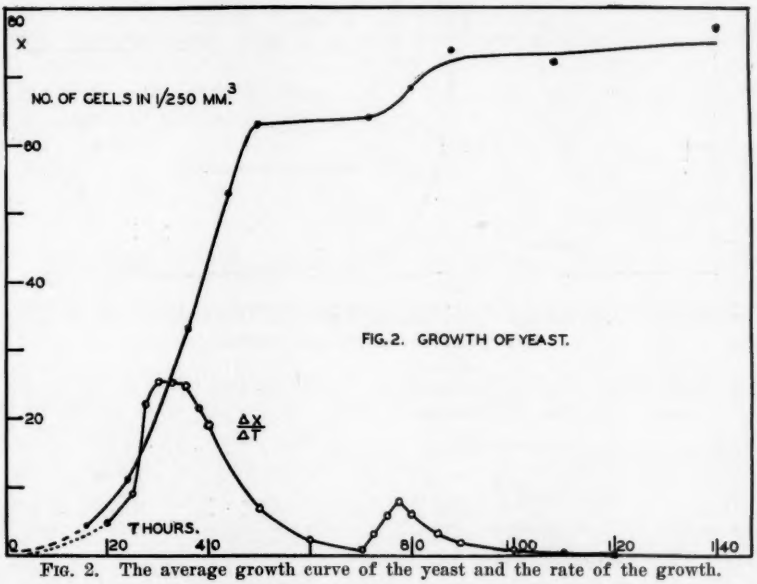
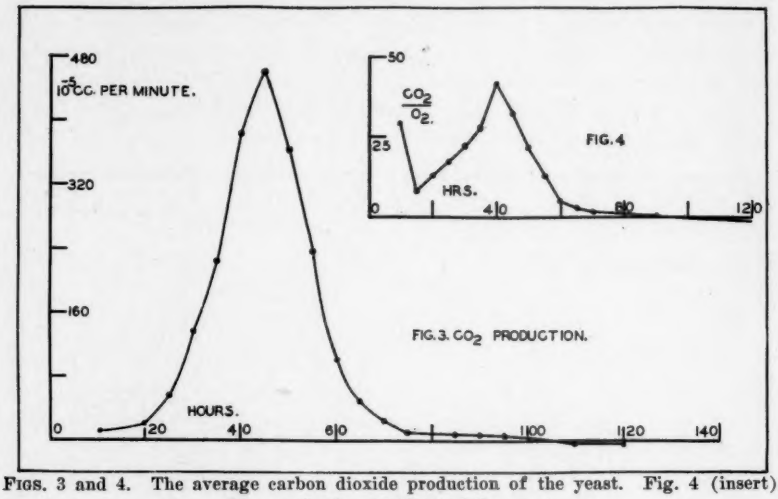


FIG. 2. The average growth curve of the yeast and the rate of the growth.

hours), and then increases to a lesser extent during the second cycle. From a comparison of the two figures there appears to be no direct correlation between the rate of growth and the rate of oxygen consumption which would suggest that the oxygen consumed is not used primarily to supply energy directly to the cells but for the oxidation of products in the medium. The oxidation of the products in the medium and the release of materials

into the medium from the disintegration of the dead cells seem to increase the fitness of the medium for the yeast. The population then increases (second cycle of growth) because less cells are killed. The percentage of cells budding shows little regular change during the two cycles of growth.[5] The oxygen uptake for the first few hours occurs gradually, indicating that the "bottom" yeast used in this investigation did not absorb the oxygen as did the "top" yeast used by BROWN (1).

A curve showing the rate of carbon dioxide production of a series of



FIGS. 3 and 4. The average carbon dioxide production of the yeast. Fig. 4 (insert). The respiration quotient of the yeast.

four experiments appears in figure 3. To obtain this curve ordinates from an average curve for the observed values of CO_2-O_2 were added to the ordinates of the corresponding O_2 consumption curve. Since the values of individual experiments are in good agreement only the average curve is plotted. The facts that the scales in figures 1 and 3 are different, and that the carbon dioxide produced greatly exceeds the oxygen consumed, must be noted when comparing those two figures.

The rate of carbon dioxide production increases rapidly until about 45 hours after seeding and then decreases until an equilibrium is reached at 70 hours. The decrease in carbon dioxide production precedes the equilibrium in growth at the end of the first cycle. The carbon dioxide probably comes largely, if not entirely, from the fermentation of sugar.[6] The yeast

[5] A detailed description of the changes in the culture medium will be published by RICHARDS.

[6] About 75 per cent. of the sugar in the medium is used up by 60 hours. (*Cf.* also RICHARDS, 7.)

used in these experiments is a bottom yeast and is growing in a medium favoring growth rather than fermentation. The products of fermentation decrease the population by selectivity, eliminating the larger buds before they can become resistant (RICHARDS, 8). Consequently, the resulting waste products negate somewhat the energy derived from the fermentation.

In view of the great amount of work on the respiratory quotient of cells and tissues it is of interest to calculate the values of CO_2/O_2 for yeast. The curve, fig. 4, plotted from these values reaches a maximum of 41.7 at 40 hours and then decreases to zero.[7] Toward the end of the second cycle glycogen is stored in the cell and the sugar in the medium is almost gone. There is, however, some change in the nitrogen content of the medium indicating metabolism of this material. This prevents any simple measure of the energy used by the cell from the oxygen entering the medium and the carbon dioxide liberated from the medium.

A series of experiments at a lower temperature (about 20° C.) indicates that the highest points on the oxygen and carbon dioxide rate-curves appear later and are reached more gradually than at 28°. The changes associated with different temperatures have not yet been sufficiently studied to warrant a more detailed comparison at this time.

Summary

1. The curves for the rate of oxygen consumption and carbon dioxide production of a population of yeast have been compared with the first two cycles of growth during 130 hours after seeding the culture. The cells were grown in WILLIAM'S medium at 28.2–28.3° C.

2. The growth rate in the first cycle in these experiments is greatest at 32 hours and the population reaches an equilibrium number at 60 hours. At about 72 hours the population of cells increases again until a second equilibrium is reached at about 90 hours. The rate of growth during the second cycle is greatest at 78 hours.

3. The rate of oxygen consumption reaches its maximum at about 65 hours after seeding during the equilibrium between the first and second cycles of growth. This suggests that the oxygen is used mainly to oxidize products in the culture medium. The rate of oxygen consumption decreases slowly and tends to reach an equilibrium at the end of the experimental period. The production of carbon dioxide is very great during the first cycle of growth (25–60 hours after seeding) but decreases as the sugar of the medium is fermented.

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[7] This is a little less than the values of 60–100 given by MEYERHOF (4), and would place this yeast intermediate between the beer and the pressed yeasts.

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EFFECT OF HOT-WATER TREATMENT ON THE CARBOHYDRATE CHANGES IN NARCISSUS BULBS DURING STORAGE

HEINRICH HASSELBRING

Introduction

Since the work of RAMSBOTTOM (**17, 18**) in England and VAN SLOGTEREN (**20, 21, 22**) in Holland, who adapted and applied the Jensen hot-water treatment for grain smuts to narcissus and other flower bulbs for the destruction of eelworms, this treatment has come into wide-spread use for the destruction of animal parasites in dormant bulbs. In accordance with the disinfection requirements which have been in effect in the United States since January 1, 1926, all narcissus bulbs imported into this country and also those entering into interstate commerce are subjected to this treatment unless, after requisite inspection, they have been found free from bulb-fly larvae and eelworms.

Aside from the destruction of eelworms and other animal parasites, European experience seems to indicate that the hot-water treatment itself has a stimulating effect on the growth of the bulbs (**23**), so that, according to VAN SLOGTEREN (**24**), this treatment, first reluctantly employed in the combating of parasites, is now annually applied in Holland to large quantities of perfectly sound bulbs. In the United States also there is a current impression among florists that hot-water treatment, which does not result in direct injury to the bulbs, brings about an increased vigor. GRIFFITHS (**6**), however, who also has noted this effect, is inclined to the belief that the favorable result is attributable entirely to the destruction of bulb mites.

Some time before the hot-water treatment of bulbs for the destruction of animal parasites came into use it had become a common horticultural practice to immerse dormant plants in warm water for short periods in order to force them into early growth and flowering (**10**). This treatment has been found by MÜLLER-THURGAU and SCHNEIDER-ORELLI (**13**) to be followed by changes in the relative quantities of the different carbohydrates within the treated tissues.

While the essence of the hot-water treatment is the same, whether applied to plants for the purpose of breaking the rest period or to bulbs for the destruction of parasites, there is a difference in the subsequent handling of the material. Plants or plant organs treated for the purpose of breaking the rest period are placed under forcing conditions immediately after the treatment, whereas bulbs which at some time during their summer dormancy are treated for parasites usually remain in storage for a time

before they are planted. It was of interest, therefore, to study the carbohydrate transformations in treated and in untreated bulbs during storage in order to determine whether the hot-water treatment of bulbs which subsequently remain dormant for a time produces on the carbohydrate constituents any persistent effect which might be correlated with the later behavior of the plants during growth.

The work was carried out with the cooperation of Dr. DAVID GRIFFITHS, Senior Horticulturist in charge of Bulb-Culture Investigations, in the Bureau of Plant Industry, U. S. Department of Agriculture. Through Doctor GRIFFITHS it was possible to obtain narcissus bulbs of constant variety and known cultural history, which were practically free from parasites and diseases.

Preparation and mode of storage of the bulbs

The bulbs used in this work were of the self-colored trumpet variety King Alfred of *Narcissus pseudo-narcissus* L. They were harvested at the Arlington Experiment Farm, at Rosslyn, Virginia, on June 28, and allowed to dry in a loft until July 8. On that day they were freed from tops, roots, and loose scales. The following day they were washed and spread out on large-meshed wire screens to dry over night in the breeze from electric fans. The next morning, July 10, the bulbs were sorted into lots of 45 each, in such manner that each lot graded as nearly alike as possible, from the largest to the smallest bulb. The lots, weighing about 2.2 kilograms each, were put into mosquito-net bags, which were spread out in a single layer on wire screens in the storage room. The next day, July 11, after the material had come into equilibrium with the room temperature, the bags were weighed. On July 12 the first set of four bags was taken to the laboratory and prepared for analysis. Every two weeks thereafter four of the remaining bags were used for analysis. All the bags in storage were weighed every two weeks in order that the carbohydrate changes might be based on the original weight of the bulbs.

On August 10, the day after the third sampling, one-half of the remaining bags was heat-treated by submergence for three hours in a stirred water-bath at a constant temperature of 43.5° C. These bags were then hung up over night to dry in the breeze from electric fans. The next day they were replaced in storage.

With the exception of slight temporary changes caused by opening the door, the storage room remained at a temperature of $17 \pm 0.25°$ C. The relative humidity ranged from 67 to 70 per cent. of saturation.

Analytical methods

In the preparation of the bulbs for analyses, the dry scales, tips, and the dry parts of the bases were removed. The bulbs were cut longitudinally

into two to six pieces, which were thoroughly mixed and ground in a meat chopper. As the dough-like mass coming from the machine could not be readily mixed or quartered it was returned to the hopper in small portions taken from different parts of the mash and reground. From the mass thus mixed, samples for analysis were weighed out. For moisture determination, samples of approximately 10 gm. were accurately weighed in weighing bottles and covered with absolute alcohol. After this had been evaporated at 60° C., the samples were dried at 70° and 16 mm. pressure in a slow current of dried air.

Twenty-five-gram samples for sugar determinations were washed into beakers with 100 ml. of 95 per cent. alcohol. Five-tenths of a gram of calcium carbonate was added to each sample. The beakers were heated to boiling for a few minutes and allowed to stand for 10 days, whereupon the alcohol was decanted into 250-ml. volumetric flasks. The residues were covered with absolute alcohol, and allowed to stand over night in a desiccator. The material thus hardened was ground in a mortar and extracted once by boiling with 70 per cent. alcohol, and for two periods of eight hours each in Soxhlet extractors.

This procedure was adopted after some preliminary experimentation during which it was found that interfering substances in the bulbs made the quantitative separation of the sugars difficult. The course of the sugar extraction in these preliminary experiments is given here. The percentages of the total extracted sugar found in the separate fractions of the extract were 58.98 in the storage alcohol; 39.90 in the filtrate from extraction by boiling; 0.60 in the first Soxhlet extract; 0.18 in the second Soxhlet extract; 0.06 in the third Soxhlet extract; 0.10 in the fourth Soxhlet extract; 0.07 in the fifth Soxhlet extract; 0.09 in a final extract made by boiling.

Each figure is the average of six determinations. In the regular work the extraction was terminated with the second Soxhlet extract.

The reductions were carried out essentially as described in a former paper (**7**).

Ten-gram samples for starch determinations were covered with 100 ml. of absolute alcohol and stored in flasks whose stoppers were sealed with paraffin. Ultimately the samples were ground in a mortar, transferred to porcelain extraction thimbles, and extracted with 500 ml. of cold 70 per cent. alcohol added in 10-ml. portions. Acid-hydrolyzable substances in the residues were determined by the acid-hydrolysis method of SACHSSE (**19**). This fraction is regarded as starch in the subsequent discussion.

All the data on carbohydrates were calculated in terms of grams of hexoses, in the equivalent at the different dates of sampling, of 100 grams of fresh bulbs at the beginning of storage. The calculation in case of each bag analyzed was based on the loss of weight of that particular bag from the

day it was placed in storage until the day of analysis. Within the limits of the sampling error the figures are therefore all directly comparable.

For the purpose of finding the shrinkage of the bulbs during storage the bags were arbitrarily divided into two lots, the odd-numbered bags forming one lot and the even-numbered the other. The even-numbered lot contained the bags later to be treated with hot water. The shrinkage for each lot was determined separately, but in each case on a decreasing number of bags, since after each weighing two of each lot were used for analysis. The results showing the shrinkage on the basis of 100 gm. of fresh bulbs at the beginning are given in table I.

TABLE I

SHRINKAGE OF 100 GRAMS OF NARCISSUS BULBS AT DIFFERENT DATES DURING STORAGE

TREATMENT OF BULBS	JULY 11	JULY 25	AUGUST 8	AUGUST 22	SEPTEMBER 5	SEPTEMBER 19
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Untreated	100	96.97	95.07	93.49	92.51	91.34
Heat-treated Aug. 10	100	97.00	95.07	*93.22	*91.36	*89.33

* These figures represent the heat-treated lots.

It is evident from these figures that after the hot-water treatment the treated bulbs lose weight at a greater rate than the untreated bulbs, so that the difference in loss becomes greater with advance of the storage season. Although MÜLLER-THURGAU and SCHNEIDER-ORELLI (13) and IRAKLIANOFF (9) found that a temporary warming of dormant plant organs results in a passing increase in respiration, the greater loss in the treated bulbs can scarcely be attributed to this effect, for the excess loss of the treated bulbs over the untreated is about two grams for 100 grams of original fresh weight, while the difference in loss of total carbohydrates is only about three-tenths of a gram.

The greater loss in the treated bulbs is, therefore, chiefly due to greater loss of water. However, the moisture determinations given in table II show that there is no decrease in the moisture content of the living tissues of the bulbs during storage under the conditions of this experiment, but rather a slight increase caused by the accumulation of water of metabolism. The actual loss of water is apparently confined to the outer scales, which after exhaustion of their contents become brown and papery. It seems that the treated bulbs lose more water in this way than the untreated bulbs.

The starch changes occurring in the bulbs during storage are given in table III, which shows that there is a gradual decrease of starch during the entire storage season. After the hot-water treatment the loss from the

TABLE II

MOISTURE CONTENT OF NARCISSUS BULBS AT DIFFERENT PERIODS DURING STORAGE

	per cent.	*per cent.*	*per cent.*	*per cent.*
July 12	67.12	67.09	66.67	66.91
July 26	66.24	66.80	66.66	66.74
August 9	67.40	68.19	67.93	67.92
August 23	67.55	67.40	*67.89	*67.24
September 6	67.30	67.37	*67.52	*67.56
September 20	67.45	67.04	*67.19	*67.15

* These figures represent lots heat-treated Aug. 10.

treated bulbs is greater than that from the untreated. The loss of starch, in terms of dextrose, for 100 gm. of fresh bulbs at the beginning of storage, is 2.55 gm. for the untreated bulbs, and 3.15 gm. for those treated in the middle of the season. These figures are based on a comparison of the average of four samples at the beginning of storage with the average of two treated and two untreated, respectively, at the end of storage. The treated bulbs, therefore, lost for 100 gm. of original fresh material the equivalent in starch of six-tenths of a gram of dextrose more than the untreated.

TABLE III

STARCH, AS DEXTROSE, AT THE DIFFERENT SAMPLING DATES, IN THE EQUIVALENT OF 100 GRAMS OF ORIGINAL FRESH BULBS

	gm.	*gm.*	*gm.*	*gm.*
July 12	23.67	24.14	24.29	24.05
July 26	24.07	23.39	23.67	23.43
August 9	23.01	22.58	22.16	22.87
August 23	22.21	22.35	*21.74	*21.86
September 6	21.58	22.05	*20.91	*21.15
September 20	21.65	21.34	*20.85	*20.94

* These figures represent lots heat-treated Aug. 10.

The transformation of starch is first reflected in the increase of reducing sugars. The changes in these shown in table IV follow a very definite trend. During the first four weeks of storage, before any of the bulbs have been treated with hot water, there is a well-marked increase in reducing sugars. Thereafter, in the untreated bulbs the reducing sugars decrease until the end of the storage season, when the reducing-sugar content is scarcely above that of the bulbs at the beginning. In the treated bulbs, immediately after the hot-water treatment, the reducing-sugar content rises and remains for some time above that of the untreated bulbs, but finally falls back to the same level as in the untreated bulbs. This observation is

TABLE IV

REDUCING SUGAR DETERMINED AS INVERT SUGAR IN THE PRESENCE OF SUCROSE, AT THE DIFFERENT SAMPLING DATES, IN THE EQUIVALENT OF 100 GRAMS OF ORIGINAL FRESH BULBS

	gm.	*gm.*	*gm.*	*gm.*
July 12	0.26	0.23	0.22	0.21
July 26	0.28	0.27	0.27	0.30
August 9	0.31	0.35	0.39	0.31
August 23	0.29	0.30	*0.36	*0.35
September 6	0.27	0.28	*0.31	*0.31
September 20	0.26	0.27	*0.25	*0.26

* These figures represent lots heat-treated Aug. 10.

in accord with the results of experiments of MÜLLER-THURGAU and SCHNEIDER-ORELLI (**13**), according to which hot-water treatment temporarily increases, under some conditions, the reducing-sugar content of *Convallaria* pips over those not treated.

Data in table V relating to sucrose changes show that increase in sucrose takes place chiefly during the last six weeks of storage coincident with the disappearance of reducing sugar. During the first four weeks there is no increase. After hot-water treatment the gain in the treated bulbs is greater than that in the untreated.

TABLE V

SUCROSE, DETERMINED AS INVERT SUGAR, AT THE DIFFERENT SAMPLING DATES, IN THE EQUIVALENT OF 100 GRAMS OF ORIGINAL FRESH BULBS

	gm.	*gm.*	*gm.*	*gm.*
July 12	3.11	3.05	3.19	3.12
July 26	3.09	3.08	3.17	3.13
August 9	3.13	3.02	3.03	3.10
August 23	3.27	3.26	*3.45	*3.59
September 6	3.47	3.54	*3.72	*3.74
September 20	3.54	3.55	*3.86	*3.86

* These figures represent lots heat-treated Aug. 10.

The carbohydrate changes in the bulbs stored under the conditions described are like those generally occurring in other starchy plant organs during dormancy. The essential feature of these changes is a gradual transformation of starch into sucrose. As is usually the case, at the relatively high temperature at which the narcissus bulbs were stored, the changes proceed slowly so that the total transformation is not great. The changes are accelerated in bulbs treated with hot water at some time during

their summer storage. The effect of this more rapid transformation is not lost, but persists until the end of the season.

The possible influence of the accelerated changes in the treated bulbs on their subsequent growth may be considered as bearing a certain relation to the accumulation of sugar in plant parts as a result of treatments that break the rest period.

Experimental results differ as to the effect of an increase of sugar, in itself, in stimulating the growth of dormant organs, but it seems evident from PFEFFER'S (**15**) critical analysis of the problem, as well as from the work of MÜLLER-THURGAU (**11, 12**) and of APPLEMAN (**1**) with potatoes, that both internal and external factors are concerned in inhibiting the growth of buds. According to MÜLLER-THURGAU'S view growth begins as soon as the internal limiting factors, withholding the sugar supply from the buds, cease to exist. The direct injection of sugar has in many cases, but not always, been found to give favorable results. HOWARD (**8**) found that buds of *Crataegus macracantha, Fraxinus excelsior, Quercus pedunculata,* and *Q. rubra* showed growth when injected with distilled water or with sugar solution. In *Crataegus* and *Fraxinus* the effect of the sugar solution was somewhat greater than that of water. Buds of *Aesculus flava* showed no growth with sugar solution, but after 27 days gave slight growth without expanding of the leaves when injected with water. Increasing the sugar content of twigs by immersing them in a two or a four per cent. glucose solution or by injecting the solutions directly into the buds was found by BORESCH (**2**) to start the buds of *Corylus avellana, Tilia grandifolia,* and *Acer pseudoplatanus* into growth earlier than those similarly treated with water. He concludes that even a slight increase of glucose in the tissues is sufficient to bring about earlier growth. DENNY, (**4, 5**), however, found that, although reagents that were effective in breaking the rest period of potatoes also increased the sucrose content of the tubers, soaking the tubers in sucrose solution or injecting it into their tissues did not hasten sprouting. That treatments which promote growth are not always accompanied by an increase in the sugar content of the treated tissues is shown by the experiments of MÜLLER-THURGAU and SCHNEIDER-ORELLI (**14**) with *Convallaria* pips and with lilacs.

Since heat treatment of resting plant organs is very generally effective in starting them into growth, it may be assumed that the primary changes leading to growth are accelerated in narcissus bulbs by the hot-water treatment. The immediately apparent result of this treatment is an increased starch hydrolysis with a corresponding increment in sucrose which persists until the end of the storage season. At the time of planting, the treated bulbs contain more readily soluble carbohydrates than the untreated ones. This greater supply of immediately available reserve food may result in

the better development of treated bulbs observed by growers, even though the stimulating effect of the hot-water treatment itself is not latently preserved, as MÜLLER-THURGAU and SCHNEIDER-ORELLI (**14**) have found with *Convallaria* pips stored subsequent to treatment. It is of interest to point out that the initial changes during storage are of the same general nature as those taking place during the subsequent growth of the plants, as shown by the investigations of CHRISTENSEN (**3**) and of MARIANNE PINKHOF (**16**) on the growth of tulips. That CHRISTENSEN could not correlate the extent to which these changes had proceeded before planting with the ease with which bulbs planted in October, December, and January, respectively, could be started into growth, scarcely bears on the question, since the bulbs at these widely separated dates could not have been otherwise comparable.

Summary

The changes taking place in narcissus bulbs stored at 17° C. may be briefly summarized as follows:

Untreated bulbs under the conditions of storage described in this paper lose about nine per cent. of their weight during a storage period of ten weeks. Bulbs heat-treated at about the middle of the storage season by immersion for four hours in water at 43.5° C. lose about eleven per cent. of their weight during the entire storage period. The greater loss of the treated bulbs probably arises from the fact that in these more of the outer scales become dry and brown than in the untreated bulbs.

Both in treated and in untreated bulbs there is a gradual loss of starch during the entire storage season. The loss becomes greater in the treated bulbs after treatment.

Reducing sugars increase gradually until about the middle of the storage season and then recede practically to the original quantity. The temporary increment is greater in the treated than in the untreated bulbs.

Sucrose undergoes scarcely any change during the first four weeks of storage, but thereafter increases until the end of the season. The sucrose content of the treated bulbs becomes greater than that of the untreated. The difference persists until the end of the storage period.

In the treated bulbs the metabolic processes are loosed or accelerated to a greater extent than in the untreated bulbs, so that at the time of planting the treated bulbs are not only more active in internal changes than the untreated bulbs, but also are supplied with a greater proportion of readily available carbohydrates. This state, it would seem, may enable the treated bulbs to get an earlier start or make a more active growth when planted.

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CATALASE ACTIVITY IN TOMATO FRUITS AT DIFFERENT STAGES OF THEIR DEVELOPMENT[1]

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(WITH ONE FIGURE)

Ever since its discovery by LOEW catalase has been the cause of much speculation and investigation. During the thirty years since its discovery several hundred papers have been written concerning it, and we are still wondering whether its sole function is that of breaking down hydrogen peroxide into water and oxygen, or whether it has other yet unknown functions to perform. Because of the already profuse literature on the subject the briefest possible statements concerning the experiments performed will be given in this paper. Readers interested in the literature are referred to the paper by BATTELLI and STERN (**2**) in 1910 and one by MORGULIS (**5**) in 1924. Each of these has a very extensive bibliography.

For several years the senior author has been investigating the growth and respiration (**3, 4**) of tomato fruits. In this connection it was thought profitable also to make some catalase determinations on similar fruits, and find out whether there was any correlation between growth, respiration and catalase activity. Hence the catalase determinations here recorded.

The method of determining the catalase activity was the same as that employed by APPLEMAN (**1**). Fruits were sliced and placed in a saturated solution of $CaCO_3$ and left there a few minutes, and then ground in a food chopper. The juice was filtered through glass wool to free it from the pulp. One cc. of this juice was placed in a bottle (the reaction chamber) and one cc. of distilled water was added to this. The reaction chamber as well as the bottles containing the juice and the container of H_2O_2 were kept at 20° C. in a constant temperature bath, which did not vary more than half a degree either way. When the apparatus had been adjusted, 5 cc. of neutral H_2O_2 was added to the reaction chamber, which was then constantly shaken by hand. Readings of the oxygen liberated were taken every minute for five minutes. The total amount of oxygen liberated for the five minutes is used in calculations throughout this report. There was of course the usual dropping off of oxygen liberation as time went on.

The fruits used for these experiments were for the most part grown in the greenhouse, though a few were grown out-doors. The experiments were conducted at four different times by three different individuals, who beforehand were not familiar with the results obtained by the others. Thus the

[1] Paper from the Department of Botany of the University of Michigan, no. 342.

results have been checked several times. All determinations were made in duplicates.

By percentage of growth is meant the percentage increase in diameter per day. This was determined by measuring with a micrometer two diameters at right angles to one another and at right angle to the axis running through the fruiting stem. In most of the experiments the fruits were measured every three or four days for a long period of time, thus obtaining a growth curve. In a few of the later experiments, the fruits were measured only twice, twenty-four hours apart, the second measurement being made when the fruits were picked. GUSTAFSON (3) has shown that diameter

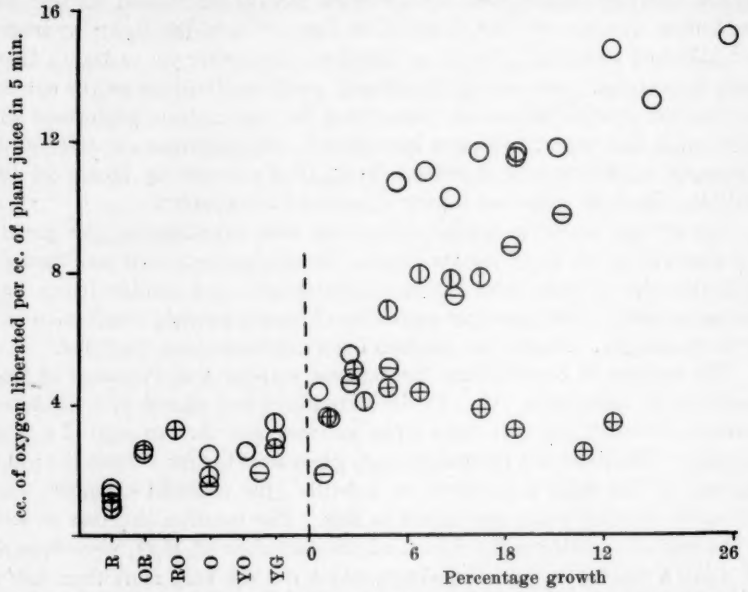

FIG. 1. This graph is plotted from the data given in tables I, II, III and IV. On the left of zero point are denoted different stages in ripeness of the fruits, while on the right of the zero are the percentages of growth the fruits made during the day preceding their picking. In designating ripeness a more or less arbitrary method has been used in noting colors: yg means yellow-green, yo yellow-orange, o orange, ro red-orange, or orange-red and r red-ripe. The figures are averages of the oxygen liberated in each group of experiments for the corresponding color or percentage of growth. The position of each point on the abscissa was determined by taking the average growth of all the fruits used in each group of experiments within ranges of every two per cent. The circles with a cross through them denote the data for the March–May, 1929, experiments, the plain circles data for the June–August, 1929, experiments, the circles with a perpendicular line through them the January–February, 1930, experiments and the circles with a horizontal line the May–June, 1930, experiments.

measurement is a perfectly satisfactory method of determining growth of a fruit. The John Baer tomato was used throughout this investigation.

The first experiments were conducted during March–May, 1929. During this period sixty-three determinations (in duplicate) were made in which sixty-nine fruits were used. The more rapidly growing fruits were quite small and several fruits were used in each determination. The data from this group of experiments are given in table I and figure 1.

TABLE I

CATALASE ACTIVITY DETERMINED MARCH–MAY, 1929

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		8	8	1.06
Orange-red		5	5	2.64
Red-orange		5	5	3.28
Yellow-green		7	7	2.73
0 – 2.0	1.13	12	12	3.65
2.1– 4.0	2.83	11	11	5.03
4.1– 6.0	4.0	4	4	4.56
6.1– 8.0	6.9	3	3	4.4
10.1–12.0	10.6	3	5	3.9
12.1–14.0	12.7	2	3	3.28
16.1–18.0	16.9	1	2	2.6
18.1–20.0	18.7	2	4	3.48

During the following June–August one hundred and seven more determinations were made in which one hundred and forty-eight fruits were used. Eleven of these experiments, using twenty-six fruits, were conducted with fruits grown out-doors. When fruits of the same growth rates are compared from the two sources there is no evident difference in the catalase activity. These determinations are given in table II and figure 1.

In January and February and again in May and June, 1930, more determinations were made. This time most of the determinations were made on rapidly growing fruits and only enough on the ripening and slow growing fruits to obtain an idea of the general behavior of these fruits. The first two groups of experiments gave different results for the rapidly growing fruits and it was thought very desirable to repeat this part of the experiments as extensively as possible. In the January–February lot, of the fifty-six fruits used thirty were growing at a rate greater than four per cent. and in the May–June lot, twenty-six out of thirty-eight were increasing at a rate greater than four per cent. for twenty-four hours, while in the first

TABLE II

CATALASE ACTIVITY DETERMINED JUNE–AUGUST, 1929

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		8	8	1.48
Orange-red		5	5	2.53
Orange		11	11	2.50
Orange-yellow		4	4	2.60
Yellow-green		13	13	3.00
0 – 2.0	0.7	19	19	4.41
2.1– 4.0	2.6	7	7	5.52
4.1– 6.0	5.7	5	5	10.81
6.1– 8.0	7.3	9	17	11.11
8.1–10.0	8.8	6	10	10.37
10.1–12.0	10.6	7	17	11.72
12.1–14.0	12.9	7	15	11.74
14.1–16.0	15.4	2	8	11.85
	18.8	1	3	14.85
19.1–22.0	21.2	2	3	13.27
	26.0	1	3	15.22

group of determinations only twenty-one out of sixty-nine grew that rapidly. The January–February and May–June determinations are given in tables III and IV as well as in figure 1.

TABLE III

CATALASE ACTIVITY DETERMINED JANUARY 31–FEBRUARY 10, 1930

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		3	3	0.86
Orange		3	3	1.85
Pink-green		3	3	3.46
0 – 2.0	1.4	7	11	3.6
2.1– 4.0	3.49	3	6	4.09
4.1– 6.0	4.91	3	6	6.95
6.1– 8.0	6.89	3	6	8.06
8.1–10.0	8.8	3	7	7.85
10.1–12.0	10.6	2	6	7.88
	12.8	1	5	11.6

TABLE IV

CATALASE ACTIVITY DETERMINED MAY 26–JUNE 4, 1930

RANGE (STATE OF RIPENESS OR PERCENTAGE OF GROWTH)	AVERAGE GROWTH IN PERCENTAGE WITHIN RANGE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF FRUITS	AVERAGE AMOUNT OF OXYGEN LIBERATED IN 5 MINUTES
	per cent.			*cc.*
Red		2	2	1.35
Orange		2	2	1.53
Yellow		2	2	1.95
0 –2.0	0.95	1	1	1.90
2.1–4.0	2.62	2	5	4.67
4.1–6.0	5.15	2	5	5.15
	8.96	1	5	7.35
	12.54	1	8	8.82
	15.77	1	8	9.80

A glance at the graph shows that the amount of oxygen liberated by the fruits growing more rapidly than 4 per cent. per day was less than that liberated by those growing between 2 and 4 per cent. in the first group of experiments. In the last three groups of experiments there was a general, though irregular, increase in amount of oxygen liberated as the rate of growth increased. In other words as growth decreased catalase activity also became less. During the ripening process there seems to be a slightly lower activity in the yellow-orange and orange fruits than in the red-orange and red ones. If the graph, with the exception of the data from the first group of experiments for fruits growing more rapidly than 4 per cent., is compared with figure 3 in the paper by GUSTAFSON (**4**) on respiration of tomato fruits, one finds a general agreement between catalase activity and respiratory rate.

We have no explanation to offer for the divergence of the results for group one experiments with fruits growing over 4 per cent. Experiments have been conducted at all times of the year for two years. The fruits have come from plants both old and young, grown in the greenhouse and outside, yet only in this group of experiments did we get this low rate of catalase activity. Within themselves these aberrant determinations agree very closely, so it does actually seem as if they were not errors of sampling, as it would be rather hard to assume that the fruits collected over a period of nearly three months could all have been of a peculiar nature different from the normal fruits. Yet it seems very strange that we should not have run into these fruits at other times, but we never have. They are included in this paper even though they seem out of place, because we do not feel that

experimental data should be suppressed merely because they do not fit in with the remainder.

In some of the fruits there is a slight indication that catalase activity is highest during the summer, but there is not enough evidence to be at all certain. From a few experiments a definite indication has been obtained that if two fruits having the same growth rate at the time of picking are used they will show a difference in catalase activity if one is still increasing its growth rate while the other is slowing down. The one that is on the ascending side of maximum growth has a higher catalase activity than the one that is past its maximum growth rate. From this one might argue that the catalase increase precedes increased growth rather than follows or accompanies it, and that catalase may in some way influence the rate of growth. This line of thought is however too dangerous to follow when based on as meager evidence as we have. It is much safer merely to state that respiration, growth and catalase activity go hand in hand and not try to assign any causal relation.

Our experiments then agree with those of some other investigators who have found that high catalase activity accompanies a high growth and respiratory rate.

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EFFECT OF BORON ON THE GROWTH OF LETTUCE[1]

J. S. McHargue and R. K. Calfee

(WITH ONE FIGURE)

The results of a number of investigations on the influence of the less common elements on the growth of plants have shown boron to be essential for the normal growth and maturation of several varieties of green plants. A definite type of injury results in some species of plants from a deficiency of this element in the medium in which the plants are grown. The quantities required for normal growth have been reported as extremely small and a slight excess is toxic for those plants which have been tested. Sommer and Lipman (3) concluded from their experiments that one-half part per million of boron is sufficient for the normal growth of most plants. They also presented evidence which shows that boron is necessary for the normal growth of a number of species of green plants. Johnston and Fisher (1) found that boron in minute quantity is necessary for the growth and the fructification of the tomato plant, and that it is required throughout the growing period, an inadequate supply resulting in retarded growth, and in injury to the fruit. The investigations of Swanback (4) showed boron to be essential for the normal growth of tobacco, a deficiency of this element resulting in a stunting of the plants, and in serious injury to the growing points. Previous work by McHargue and Shedd (2) showed that boron is essential for the growth of oats.

The effect on the growth of lettuce produced by a lack of boron was investigated by growing plants in a medium free from this element. The medium was prepared by mixing purified basal mineral nutrients with purified sand in the following proportions:

Purified sand (SiO_2)	1000.00 Gm.
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 "
Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$)	0.75 "
Potassium nitrate (KNO_3)	0.5 "
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.5 "
Potassium chloride (KCl)	0.25 "
Potassium sulphate (K_2SO_4)	0.5 "
Hydrous ferric oxide ($Fe(OH)_3$)	1.0 "
Distilled water (H_2O)	200.0 "

Each of these chemical compounds was tested qualitatively and found to be free not only of boron, but also of manganese, copper, zinc, cobalt,

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. Published by permission of the Director.

nickel, barium, strontium, lithium, and arsenic. The sand was prepared by digesting it with hot hydrochloric acid in a large porcelain dish and then washing it free from chlorides. The distilled water used for the growth of the plants was condensed in a quartz tube. Acid-proof pots which had been previously washed with hydrochloric acid, autoclaved, and rinsed with distilled water were used in growing the plants.

For experimental cultures, five kilograms of the purified basal medium was placed in each pot. The total weight was recorded and the moisture content kept at 20 per cent. through the growing period by the addition of distilled water until the required weight was reached.

Six pots containing six plants each of Big Boston head lettuce were included in the first experiment. A good germination of the seeds was obtained and a rapid growth was made by the young plants for the first two weeks. Growth during the third week was slower, and towards the latter part the newer leaves were noticeably lighter in color. The plants at this time had produced 10 to 12 additional leaves. At the beginning of the fourth week a few dark spots appeared on the tips of the younger leaves. These spots continued to increase in size and number, spreading along the margin of the leaves. All plants from which boron was withheld were affected in this manner. In a few days after the appearance of the spots the growing points died and turned black, giving the plants a badly burned appearance. The younger leaves continued to die, and in a few more weeks the entire plant was dead.

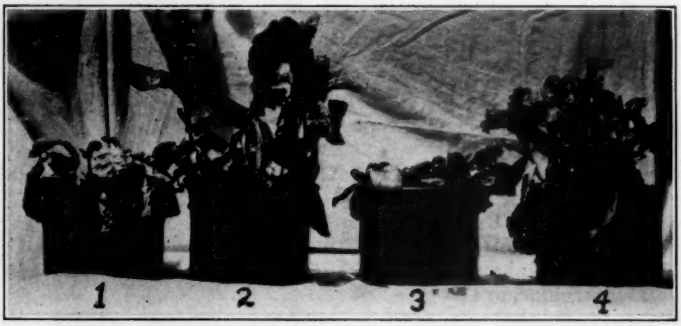

FIG. 1. The effect of boron on the growth of lettuce, other less common elements being present. Pots 1 and 3 contain no boron. The cultures in pots 2 and 4 received 0.0025 gm. of boron. Leaf lettuce, Black-Seeded Simpson, is in pots 1 and 2, and Big Boston head lettuce is in pots 3 and 4.

The presence of the disease at the same stage of growth in all plants indicated a nutritional deficiency rather than a disease of bacteriological

etiology. Microscopical examination of the tissue failed to reveal any pathogenic organisms.

A second experiment consisted of negative control cultures, containing only the purified basal mineral nutrients; positive control cultures containing, besides the basal minerals, small amounts of manganese, copper, zinc, iodine, arsenic, and boron; and cultures containing only the basal minerals and boron. Big Boston head lettuce was again used as the experimental plant.

The growth of the negative control plants followed closely that of the plants in the first experiment. At the time the negative control plants developed the spots on their leaves, all plants receiving boron were normal and were considerably larger and greener in color. At the time all of the plants in the negative control cultures were dead, no plant receiving boron had developed any symptoms of any nutritional deficiency.

A third experiment with twelve pots of leaf lettuce (Black-Seeded Simpson) showed this plant also to develop the characteristic leaf injury when boron was absent. Treatments of various combinations of manganese, copper, zinc, nickel, cobalt, barium, strontium, iodine and arsenic failed to improve the condition. After the growing points of all plants were dead, a small amount of boron (0.005 gm.) in the form of calcium borate was added to each of six of the pots. Improvement was observed on the third day after this treatment. Dormant buds in the leaf axils started growth in all boron-treated cultures, and only in boron-treated cultures. The new leaves produced were normal in appearance. Rapid growth was made in all pots, and finally stalks which produced seeds developed. The plants in pots not treated with boron died, regardless of treatment with other elements. Other varieties of lettuce tested with similar results were Iceberg and Mammoth Black-Seeded Butter. These varieties, also, were found to burn unless boron was present.

Summary

1. Three varieties of head lettuce and one of leaf lettuce required boron for normal growth.

2. The deficiency of boron in cultures causes a burning of the leaves, and the death of the growing points of head and leaf lettuce at an early stage of development.

3. The function of boron in the metabolism of the lettuce plant is not performed by manganese, copper, zinc, nickel, cobalt, barium, strontium, iodine or arsenic.

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MOVEMENT OF ORGANIC MATERIALS IN PLANTS: A NOTE ON A RECENTLY SUGGESTED MECHANISM[1]

F. C. Steward and J. H. Priestley

A recent paper by Crafts (1) discusses in an interesting manner the mechanism which effects rapid movement of organic solutes in the tissues external to the cambium. That such rapid movement, usually in a downward direction, does actually occur few will question. The general magnitude of sugar and also of organic nitrogen movement has been estimated by Mason and Maskell to be of the order of physical diffusion in gaseous systems rather than the much lower rates, possible by diffusion alone in aqueous systems. Such a problem clearly demands careful scrutiny of any theory regarding a possible physical mechanism, especially one elaborated with due regard to the histological nature of the tissues concerned. Crafts suggests a mechanism very similar to that of Münch (3) but is led by certain interesting anatomical observations to suggest that the translocation proceeds not in the sieve tubes (the most striking, longitudinally specialized cells of the phloem) but principally along the cell walls of the whole phloem tissues including sieve tubes, companion cells and parenchyma. The mechanism is not analogous to diffusion as Mason and Maskell propose but is definitely dependent upon mass flow of solution in the cell walls. Crafts develops his theory as the result of a laudable attempt to decide anatomically the route offering least resistance to mass flow. The writers have every sympathy with such an anatomical approach but are equally clear that any apparent conclusions indicated on purely anatomical grounds must be interpreted strictly in accordance with physical principles and probability. We welcome the interesting contributions which Crafts has made to the histology of the phloem but it is the purpose of this note to show that some of the interpretations fundamental to the mechanism suggested are physically inadmissible.

Crafts' rejection of the sieve tubes as the path for translocation in favor of the cell walls is a distinctly novel feature. This follows principally upon the interesting observation that the total cross-sectional area of the phloem mounted fresh is composed of cell wall material in much greater degree than would be expected from preparations fixed and mounted in the usual manner. In the latter case it is shown that marked shrinkage of the wall occurs. The relative contributions of sieve tubes, pores in sieve plates, and the walls of all the phloem cells to the cross-sectional area of undried phloem were estimated by projection methods. Crafts arrives at the con-

[1] From the Department of Botany, University of Leeds, England.

clusion that, with due regard to the total area of sieve tubes, the presence of frequent sieve plates with small pores, and the hitherto unsuspected area of wall substance, the latter route would be the more efficient. This conclusion is justified on two principal grounds: (1) The calculated pressure gradient required to transmit a probable sugar solution by mass flow along the sieve tubes at a rate sufficient to ensure the observed transfer of dry matter (a) along a potato stolon, (b) in the stem of *Cucurbita,* (c) out of a leaf, are large (of the order of 20 atmospheres per meter). Similar calculations indicate that the corresponding pressure gradients necessary to effect transfer along the cell walls of the phloem are quite small (of the order of 1×10^{-6} or 6×10^{-5} atm. per cm. and actually as low as 1.6×10^{-8} atm. per cm. to secure transfer in a tree). (2) It is suggested that exudation studies on cut phloem tissues indicate normal mass flow in the walls of the phloem of an order quite adequate to secure the observed transfer of organic nutrients.

The most important argument is that based on the required pressure gradients along the sieve tube and walls respectively. The necessary calculations were based on the familiar Poiseuille relation between the rate of flow and the pressure gradient along a tube of uniform cross-section, the viscosity of the solution, and the dimensions of the tube under conditions of uniform, non-turbulent flow. It is the purpose of the writers to show that the use made of the Poiseuille relation is such as to arrive at quite incorrect conclusions. CRAFTS arrives by a rather devious series of calculations at a figure for the pressure necessary to cause a mass flow at the required rate through the sieve pores of *Cucurbita.* In spite of the fact that the formula used is for uniform horizontal flow through a capillary this figure no doubt represents the order of pressure gradient required (20 atmospheres per metre). However, it is compared with a pressure calculated to represent that necessary to cause a mass flow at the required rate along the walls of the total phloem. It may be stated definitely that this pressure (1.5×10^{-5} atmospheres per cm.) has no relation whatsoever to the true pressure involved. The reason is fairly obvious. In this, as in all other cases where CRAFTS calculates pressure gradients necessary to cause mass flow along walls, he treats the total phloem wall area (or in the case of *Cucurbita* the wall area per bicollateral bundle) as a single pore. By various means this (the total wall area) was derived, and the radius of the equivalent circular area determined (see p. 18 and also p. 14, etc.). This radius was then inserted in the Poiseuille relation in which r can legitimately equal only the radius of a single capillary, thus:— $P = \frac{8Rnl}{\pi r^4}$.

where P = pressure in dynes per sq. cm.
R = rate of flow in cc. per sec. through total phloem walls.
n = viscosity of a 10 per cent. sugar solution.
l = length of gradient or tube in cm.
r = radius of conducting element due to the walls of which the shearing forces are exerted.
A = area of capillary through which flow takes place.
R_1 = linear rate of flow.

After CRAFTS:

$$R = R_1 \times A$$

$$P = \frac{8R_1 \times A \times n \times l}{\pi r^4}$$

$$P = \frac{8R_1 \times \pi r^2 \times n \times l}{\pi r^4}$$

$$P = \frac{8R_1 nl}{r^2}$$

where r = radius of circle equivalent to total phloem wall.

Correct:

$$R = R_1 \times A \times N \qquad A = \pi r_1^2$$

$$P = \frac{8R_1 \times A \times n \times l \times N}{\pi r_1^4}$$

$$P = \frac{8R_1 \times \pi r_1^2 \times n \times l \times N}{\pi r_1^4}$$

$$P = \frac{8R_1 \times N \times n \times l}{r_1^2}$$

where r_1 = radius of a single, circular, conducting element.
N = number of such capillaries in total area through which flow proceeds.

The resistance to flow through small pores depends on the shearing forces which are the result of steep velocity gradients in the moving liquid. The liquid in contact with the pore wall is theoretically at rest and that farthest from it has maximum velocity. In the Poiseuille expression for steady flow through a horizontal uniform circular capillary r represents the distance separating liquid surfaces at rest from that moving with maximum velocity. By utilizing in Poiseuille's expression the radius of the circular area equivalent to the total phloem area and a derived linear rate of flow, CRAFTS in effect says that the theoretical liquid surface at rest in contact with the pore wall is separated from that of maximum velocity by a relatively large length—the radius of the circle equivalent in area to the total phloem wall—whereas if flow did take place through the walls, it is clear that the true value should be of the order of half the mean wall thickness. In short, CRAFTS found the radius of the *actual pores* through the sieve plate, but when dealing with flow through the whole wall used a pore radius of a wrong order of magnitude. Since the formula demands $\frac{1}{r}$ to the fourth power it is clear that the derived pressures are of a totally wrong order of magnitude.

From the preceding formulae it is seen that one ought to multiply CRAFTS' derived pressures for mass flow through walls by a quantity $\frac{r^4}{r_1^4}$, since N obviously $= \frac{\pi r^2}{\pi r_1^2}$. This factor which equals the $\left(\frac{\text{radius of the equivalent single pore}}{\text{radius of the true, circular capillary pore}}\right)^4$ is large. If flow assumed through the whole wall is to be calculated as through circular pores at all,

it would seem legitimate to state that r_1 at its maximum must be of the order of half the mean wall thickness. From CRAFTS' projection figures and magnification factors this can be estimated and r is known. It is thus possible to show that, whereas CRAFTS calculated that very small pressures would be adequate to cause the required mass flow along walls these are really of the general order of 10^6 times too small. For example, in the case of the potato stolon the value of 1.1×10^{-6} atmospheres per cm. is more probably of the order of 78.2 atmospheres per cm. Similarly the small pressure gradient of 1.5×10^{-5} atmospheres per cm. calculated as necessary to cause adequate mass flow through the walls of the phloem of *Cucurbita* becomes 11.89 atmospheres per cm. The same argument applies to all the pressure gradients calculated for flow through phloem walls. The bigger the area of the single pore used by CRAFTS (*i.e.*, the area of total phloem wall) the more improbable do his pressure gradients become. It will be noted that these more probable pressures assuming flow through the whole wall are either numerically greater than, or of the same order of magnitude as, those estimated to secure mass flow through the sieve pores. The conclusion, therefore, that the pressure gradients necessary to secure flow through walls are much less than those required to cause mass flow through the sieve pores, is on these grounds alone unsound. It is true that CRAFTS does not suggest that the total phloem wall is really a single pore, but he regards the factor of 4400 which represents the discrepancy between his derived pressure and that gradient which he conceives to be probable, as sufficient safeguard against the "walls acting as a single capillary and acting as they do in the plant"—(*l.c.*, p. 20).

It is clearly unsatisfactory to use the expression for flow through circular pores at all. The exact calculation for flow along a system of the type of the phloem wall would be difficult to compute. One can, however, estimate the pressure gradients necessary to cause the required mass flow along the phloem wall were this straightened out to form a single sheet bounded by parallel plane surfaces separated by the mean wall thickness. For uniform viscous flow of a liquid of viscosity (n) between parallel planes, the pressure gradient per cm. $\left(\frac{\partial p}{\partial y}\right)$ is given in terms of the volume rate of flow per sec. (V) across unit width (h sq. cm.) between planes h cm. apart by the formula (2): $V = -\frac{h^2}{12n} \times \frac{\partial p}{\partial y}$ $\quad V = Uh$ where U = linear rate of flow.

$$U = -\frac{h^2}{12n} \times \frac{\partial p}{\partial y}$$

For the case of *Cucurbita* (p. 18) using U = 0.0759 cm. per sec. (not 0.045 as CRAFTS[2] calculates)

[2] CRAFTS derives the mean linear rate by dividing the mean of a series of total flows by the mean of a series of areas. He ought to work out the linear rates individually and find the mean of these. (See top, p. 16.)

$$h = \frac{0.25}{400} \text{cm. estimated from Plate Va}$$

$$n = 0.015$$

$$\frac{\partial p}{\partial y} = 3.50 \times 10^4 \text{ dynes per sq. cm. per cm. length}$$

$$= 0.035 \text{ atm. per cm. approximately.}$$

Pressure gradient per metre = 3.50 atm. approximately.

This value, which assumes in effect that the moving liquid is only sheared in a direction at right angles to the wall surface, is perhaps a minimum one and is of an entirely different order of magnitude from that calculated by CRAFTS. Further analysis of the application of the Poiseuille expression to mass flow of solution through the whole of the phloem walls seems superfluous since it is obvious that the whole wall cannot admit of mass flow. The actual pores are probably sub-microscopic and of the order of intermicellar spaces, *i.e.*, quantities to be measured in Ångström units. Without attempting further computation it is obvious that with this in mind even the preceding pressure gradients themselves of the order of 10^6 times those utilized by CRAFTS to support his thesis are really much too small. It is clear that CRAFTS himself realizes this point for in his discussion he says: "The fact remains that the spaces through which it is proposed that food materials in solution are passing are of molecular dimensions and irregular in form. Since no formulae are available by which to calculate pressure gradients and rates of flow through this type of material mathematical treatment is impossible. Use of Poiseuille's formula leads to high values fully as great as the ones which appeared on the sieve tube calculations."

In spite of this the whole case that the actual flow is through the walls depends for its justification upon the pores through which the flow takes place being not only much greater than the visible sieve plate pores but actually greater than the wall thickness itself. The probable pressures to secure mass flow through the whole wall are certainly either of the order of, or greater than, those calculated for flow through the sieve pores whose actual diameter was computed. From the molecular constitution of the cell wall as now known the presumption is that a mass flow could proceed more readily through the sieve tubes with their visible pores than along the phloem walls.

Since the application of the Poiseuille expression to the mass flow of solution along the phloem does not really justify the conclusion (as CRAFTS claimed) that the movement of organic solutes is along the walls, is there other evidence which does support such a novel idea? CRAFTS utilizes the well-known fact that the phloem of *Cucurbita* exudes droplets of sap as evidence of normally occurring mass flow in the phloem. From measurements of total phloem wall and also of sieve tube and sieve pore areas in

the plants concerned he concludes by arguments identical with those already criticized that this flow takes place in the walls and not in the sieve tubes. We wish, however, to draw attention to the data which indicate that the rate of such flow, as determined by exudation studies, would be adequate to effect translocation into cucurbit fruits. The linear rate of flow presumed to be in the phloem walls was determined from the volumes collected in a given time. However, the flow was never observed for a period *longer than five minutes.* Where consecutive collections were made from one stem after a single cutting, the rate observed steadily decreased with time and stopped after five or six minutes (see table VII). In fact to make several collections from a single stem it was necessary to cut the stem repeatedly. This is ascribed to plugging of the cut surface by proteinaceous material which gelatinizes upon exposure to air. It seems much more probable that in a succulent plant of the type used the exudation from the phloem is not an indication of a normally occurring mass flow at all, either in the walls or sieve tubes, but may be due rather to the turgor of surrounding tissues and the release of tissue tensions consequent upon cutting. A new cut then merely opens up a new length of stem. However, apart from such a criticism, drastic as it is, CRAFTS is surely not warranted in comparing the rate of flow in cm. per min. observed over a period *not exceeding five minutes during which constant values were never attained* with the rate of flow calculated as necessary to account for uptake of dry matter by a pumpkin. In the latter case the rate is assumed uniform *over a period of 100 days* and the rate is calculated as cm. per min. Allowing such latitude in the choice of time intervals and such tolerance of what constitutes a steady flow can produce only purely fortuitous arithmetical agreement of results.

It is not our purpose to discuss the basic principle of the mechanism suggested by MÜNCH and CRAFTS, interesting as it is. We merely wish to point out that whether or not the phloem walls do form an important avenue for translocation, the data and argument used by CRAFTS to arrive at this conclusion are unsatisfactory, and ought logically to lead to a different one. The interesting fact remains that phloem *in vivo* has apparently much thicker, hydrated walls than we have hitherto supposed. That, however, does not necessarily indicate that they function in translocation over long distances. The application of formulae from classical physics, which apply usually to well defined cases which are rarely, if ever, satisfactorily duplicated in the living plant, involves often the adoption of arbitrary assumptions which may vitiate the conclusions drawn. We feel that in this particular case the utilization of the Poiseuille relation is particularly unfortunate and has led to entirely erroneous conclusions.

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BRIEF PAPERS

CONTAINER FOR THE STUDY OF THE BEHAVIOR OF INDIVIDUAL ROOTS[1]

(WITH ONE FIGURE)

In the course of studies on the water relations of pineapple plants it became necessary to measure the water absorption efficiency of individual roots. A container (fig. 1) was then constructed which served its purpose very well and also proved very useful for various other physiological studies. A description of the construction of this container and its operation is given so that other investigators interested in root studies may profit.

The construction of the container consists of four perforated detachable sides and a bottom, all of which are fastened to a framework either by means of screws or hooks. The container may be constructed either of wood or metal, the former being preferable for certain purposes and the latter for

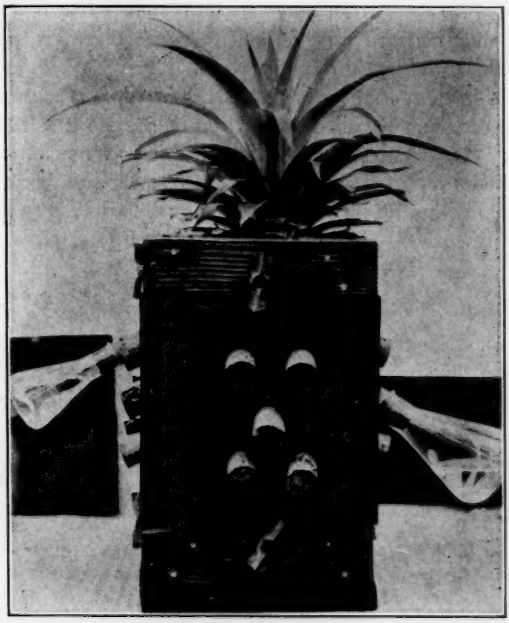

FIG. 1. Container for the study of the behavior of individual roots.

[1] Miscellaneous Paper no. 9 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

others. It may be constructed in different sizes according to the size of the root system of the plants under experimentation.

The size of the container most suitable for our work measures 6 inches in width by 6 inches in depth and 8 inches in height. The substratum employed for the germination and subsequent growth of the plants in this container is river sand. After the plants have germinated and are growing the sides are opened at 2-day intervals and examined for roots. If such roots are in sight, proper connections are established by means of glass tubes between the root and outside container, a test tube or a flask, containing either a nutrient solution or soil. The glass tube, of an appropriate size, is connected to two rubber or cork stoppers, one of which is pointing towards the side of the container and the other one towards the flask containing the nutrient solution. The root is led into the glass tube very carefully and allowed to continue its elongation without any further disturbance. The vapor pressure of nutrient solution in the outside container is sufficient to maintain the root tip moist while in the glass tube and before reaching the solution proper. After the nutrient solution is reached, quantitative determinations of water absorption may be conducted. The results which one obtains by this method are very reliable, because there are no water losses other than those of absorption by the roots.

After the isolated roots become well established, further watering of the sand may be discontinued and the nutrition of the plant left entirely to the isolated roots. One can easily determine in this way not only the efficiency of individual roots for water absorption but also the number of roots which a plant must have at different periods of growth to carry on its normal physiological processes.—C. P. SIDERIS, *University of Hawaii, Honolulu, T. H.*

A SIMPLE DILATOMETER

(WITH ONE FIGURE)

The dilatometer here described was designed to measure accurately the changes in volume accompanying imbibition. The method has been used previously by HAMPTON (**1**), SVEDBERG (**2**), and others in studying the hydration of colloids. The dilatometer we have used in our work possesses certain advantages that facilitate its use and increase its accuracy.

The instrument consists of the usual bulb surmounted by a capillary tube in which volume changes are observed. There is an additional outlet with a stopcock for regulating the initial volume, and a mercury well or trap to keep the material being studied apart from the water until the beginning of the experiment. The dimensions given in the following description apply to the instrument we have used, but they may be modified as the materials or aims of the worker require.

As indicated in the accompanying figure the dilatometer consists of a heavy-walled glass bottle (500 ml.) fitted with a ground glass stopper. The lower half of the bottle is filled with some suitable cement except for a cylindrical well in the center. This well is conveniently formed by cementing a two inch piece of large bore (1–$\frac{1}{8}$″) glass tubing to the center of the bottom of the empty bottle and filling the space between the tubing and the bottle with cement. The cement is sloped upward from the rim of the well to the walls of the bottle. The cement used must be unaffected by water and must adhere firmly to the glass on cooling. We have found "Picein" satisfactory for this purpose, although other cements may be equally as good.

The ground glass stopper is drilled with two holes spaced opposite to each other just inside the flange of the stopper. A convenient drill for this purpose may be made by fastening a short piece of copper tubing of the desired diameter to the shaft of an electric motor with a piece of heavy-walled rubber pressure tubing. With ordinary valve grinding compound as an abrasive, such a drill will penetrate a thick glass stopper in a few minutes. A meter length of capillary tubing is inserted in one hole and a short stopcock tube is placed in the other. These tubes are held firmly in place by filling the hollow stopper with hot cement. Care must be taken to have the tubes and the cement level with the bottom rim of the stopper.

A lead weight (fig. 1, C) coated with cement is provided to hold the material beneath the mercury until the start of the experiment. Two parallel wires are cemented to the base of the weight to act as supports, and to hold it enough above the rim of the well to permit the introduction of mercury and the removal of the air during evacuation. A helical spring slightly smaller in diameter than the well is cemented by means of a short

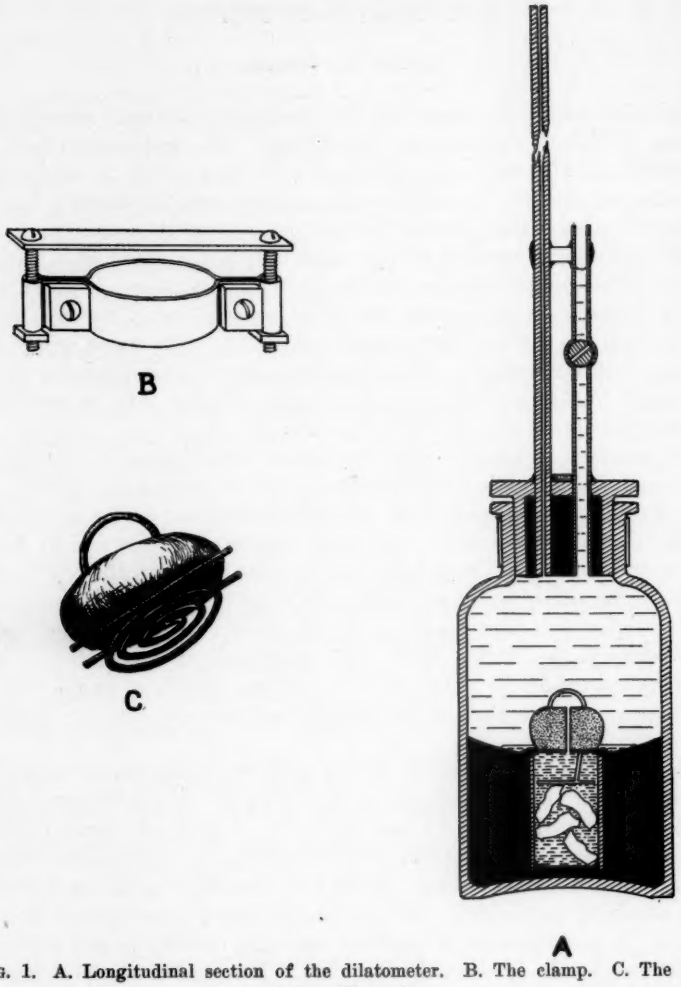

FIG. 1. A. Longitudinal section of the dilatometer. B. The clamp. C. The lead weight with spring.

wire to the base of the weight. This serves to hold the material beneath the mercury during the evacuation of the air and other preliminary operations. A small wire ring is fastened to the top of the weight to facilitate its manipulation in the bottle.

A clamp (fig. 1, B) is necessary to hold the stopper firmly in the neck of the bottle. This consists of a bar across the top of the stopper fastened with screws to a copper band around the neck of the bottle.

To operate the instrument the material being studied is placed in the well and the lead weight placed on top. Mercury is then added to more than fill the well and the bottle is evacuated in a chamber to remove all air from the mercury and material. After evacuation the bottle is filled to the top of the neck with distilled water that has been boiled to remove air. The water may be introduced as a fine stream through a glass nozzle by means of a siphon. If the glass nozzle is kept beneath the surface of the liquid, aeration of the water is avoided. The jet of water may be manipulated as the bottle is being filled to prevent the formation of air bubbles on the walls of the bottle or on the surface of the weight. When completely filled, the stopper is slipped into place in such a way as to force the water into both the capillary and stopcock tubes. The stopper must be firmly seated and clamped snugly in place. The bottle may again be evacuated through the capillary if desired, though for most purposes this is unnecessary. After adjusting the level of the water in the capillary tube to the desired height through the stopcock tube, the bottle is submerged in a constant temperature bath until equilibrium is attained. This is indicated by the constancy of the level of the meniscus in the capillary tube.

During these operations the colloidal material has been held beneath the mercury. To start the experiment the bottle is tilted slightly, allowing the weight to roll off the well and permitting the material to rise into the water. Readings of the height of the meniscus in the capillary taken at intervals will enable the operator to follow the progress of the hydration as closely as desired.

For use with powdered materials a small cup of glass, porcelain, wax or other suitable material may be used as a container. The cup is filled with the material which is held in place with a light plug of glass wool. The cup is then put into the well and the weight set in place as before.

We have found it desirable to run a simultaneous blank determination with water and mercury alone to permit corrections for minor changes in temperature. Because of the size of this particular dilatometer, variations of less than 0.1° C. in the temperature of the bath produced significant fluctuations in the level of the meniscus.

The instrument has proved to be satisfactory for research work requiring a high degree of accuracy and as a convenient method of demonstrating to classes the very considerable condensation of water accompanying imbibition.—D. B. Anderson, *Department of Botany*, and C. F. Williams, *Department of Horticulture, North Carolina State College, Raleigh, North Carolina.*

1. Hampton, H. C. A dilatometer for measuring the hydration of colloids. Science n.s. **63**: 49–51. 1926.
2. Svedberg, The. Density and hydration in gelatin sols and gels. Jour. Amer. Chem. Soc. **46**: 2673–2676. 1924.

NOTES

New Orleans Meeting.—The eighth annual meeting of the American Society of Plant Physiologists at New Orleans was in certain ways the most important one held since the organization of the Society. The Executive Committee had considered many problems pertaining to the development and welfare of the Society and brought them before the annual meeting for action.

Included among these matters were the report of the committee which has been studying the advantages and disadvantages of incorporation; insertion of regulations for the life membership awards into the constitution; allocation of additional funds for the Secretary's office; and assistance in the editorial office. Other important matters have been pressing for consideration. More information in regard to the action taken at the annual meeting will be presented in the April number of PLANT PHYSIOLOGY.

Summer Meeting.—A summer meeting of the American Society of Plant Physiologists jointly with the Corn Belt Section of the American Society of Agronomy is being arranged, the meeting to be held at the University of Wisconsin, Madison, Wisconsin, probably in July, 1932. It is hoped that many of our members from the middle western states will keep this meeting in mind, and plan to attend it. The details of the arrangements will be included in the April number of PLANT PHYSIOLOGY.

The Sachs Anniversary.—The one hundredth anniversary of the birth of JULIUS VON SACHS, which falls on October 2, 1932, will be commemorated by botanists throughout the entire world. The American Society of Plant Physiologists has dedicated the 1932 volume of PLANT PHYSIOLOGY to SACHS in honor of this event. Steps are being taken to make the meeting at Atlantic City in December, 1932, a SACHS Anniversary meeting. We are happy to be able to present to our members the characteristic portrait of SACHS as a frontispiece to the volume. During the year an interior view of his famous laboratory, and other commemorative photographs will be reproduced, to revive and recreate memories of one of the greatest botanical leaders during the last half of the nineteenth century. His work has exerted a profound influence upon the development of botany during the last sixty-seven years, and will continue undoubtedly for many decades as one of the major forces in determining the trends of botanical research.

Membership.—During 1931 the publication of 1,000 volumes of PLANT PHYSIOLOGY cost practically the entire allotted income of the journal. The accumulated reserves and sale of back volumes will make possible some of

the financial changes which the Executive Committee are recommending to the Society for adoption. Future increases in the size of the volumes published must be contingent, however, upon increased income. Fortunately the present income makes possible a very substantial volume. But the membership and subscription lists can be increased in numbers if each member will take a personal interest in offering the opportunity of membership to others whose work will be enriched and enlarged by possession of a personal copy of the journal for constant use. The Secretary will appreciate the assistance of all members in this enterprise of increasing the membership and library support of the Society.

Bibliography of Low Temperature Relations of Plants.—No good method of supporting the publication of bibliographies has as yet been found. Some time ago the Department of Agriculture of the University of Minnesota sent out an announcement of the preparation of an annotated bibliography of the low temperature relations of plants by Dr. R. B. HARVEY, who has spent much time in research in this field, and a great deal of time in accumulating the data for the bibliography. Publication, however, is contingent upon receipt of a sufficient number of advance subscriptions. In order to guarantee the costs of publication it will be necessary to secure about 500 subscriptions at $2.00 each. The estimated size of the volume is about 200 pages. It would probably be issued in stiff paper binding. Those who would like to help insure the publication of this volume can do so by writing to the Division of Publications, University Farm, St. Paul, Minnesota, and asking them to enter an order for one or more copies. Libraries might also be requested to order a copy conditional upon the receipt of a sufficient number of orders. Cooperation of physiologists in this way is about the only solution to the problem of publication of bibliographies.

Plant Physiology.—The second volume of Dr. S. KOSTYTSCHEW'S Lehrbuch der Pflanzenphysiologie has been published by Julius Springer, Berlin. It is very fortunate that the work was completed while KOSTYTSCHEW still lived. The work will be his chief monument. This volume was shared with Dr. F. A. F. C. WENT, who wrote the chapters on growth and movement. The chapters by KOSTYTSCHEW are on the fundamental physiology of intake and translocation of materials, absorption and excretion, water relations of the plant, movement of plant sap in the vessels, and translocation and distribution of nutrients in the plant. The work taken as a whole is excellent, and deserves careful reading. The closing chapters by WENT are most difficult to handle. Plant physiologists in America will appreciate

having these chapters written by the director of the Utrecht laboratory, where the most recent outstanding studies of plant movements have been made. No one engaged in research on the physiology of plants will want to omit this work from his ready reference library. It contains 459 pages, with 72 text figures. The price of the work bound in paper in 28 RM., and 29.8 RM. bound in cloth. Address Julius Springer, Linkstrasse, 23–24, Berlin W9, Germany.

Zonation.—A second edition of Dr. ERNST KÜSTER's Über Zonenbildung in kolloidalen Medien has been published by Gustav Fischer, Jena. The first edition was published about 18 years ago, so the two editions differ greatly. The first chapter considers the phenomena described by LIESEGANG many years ago, which are often referred to as Liesegang rings. Chapter two deals with formation of sphaerocrystals and lamellated membranes. The third chapter takes up membrane thickenings, such as are found in the vascular elements, and lattice formation. The last three chapters are devoted to the color markings of leaves, flowers and seed coats, growth rings of dicotyledonous plants, and fairy rings of the fungi. The book thus brings together quite diverse phenomena which only by a great stretch of the imagination can be linked up with common causes. There is surely nothing in common between the annual growth rings of a tree and the true Liesegang periodic precipitation rings. Eight pages of literature citations and an index complete the volume, which contains 124 pages. The price of the volume in paper binding is 8 RM.

Experimental Plant Physiology.—A small book by Dr. G. J. PEIRCE, of Stanford, presents a running account of an experimental approach to the problems of plant physiology. While some directions are given for laboratory experiments, it is not a laboratory manual in the usual sense. It might serve as a basis for a well planned informal series of exercises, leaving much of the details of instruction with the person in charge of the class. The experiments are described in ten chapters, as follows: The properties of cells; absorption; the movement of water; the manufacture of food; the storage of foods; digestion; oxidative metabolism; growth; irritability; and reproduction. With index it contains 165 pages. The publishers are Henry Holt and Co., New York, who quote a price of $1.50 per copy.

Colloid Chemistry.—The third volume of JEROME ALEXANDER's Colloid Chemistry has been published by the Chemical Catalog Co., New York. The papers included in this book make up the first series of papers on technological applications of colloid chemistry. There is to be a second series of these

papers in the fourth and final volume of the set. Volume III contains 42 papers, most of which are of greater value to students of physical and chemical sciences than to biologists. The last four papers, however, are of interest in biology. Three of these deal with colloidal properties of soils, and the final one with the colloidal properties of wheat, wheat flour, and wheat flour products. The book contains 655 pages, and is priced at $10.50 by the publishers.

Introduction to Cytology.—Dr. L. W. SHARP's Introduction to Cytology has been revised and translated into German by ROBERT JARETZKY, Professor of Pharmacognosy in the Technical High School, Brunswick. Several hundred papers have been considered in the revision, and certain topics such as protoplasmic inheritance, conversion of genes, mitogenic rays, etc., have been given a place in the translation. There are 733 pages, including the index, and a literature list of 72 pages. The price of paper bound copies is 52.5 RM., and of cloth bound copies, 55 RM. Orders for this work should be sent to Gebrüder Borntraeger, Schöneberger Ufer 12a, Berlin W35, Germany.

A Shorter Course in Organic Chemistry.—Every plant physiologist is confronted with the necessity of keeping abreast of the developments in organic chemistry as a part of the background of plant physiology. A few books on organic chemistry must be kept at hand for consultation. Dr. J. C. COLBERT of the University of Oklahoma has prepared a foundation work which ought to be useful to those needing a short and elementary introduction to this fascinating field. It contains many useful diagrams, especially diagrams of the types of reactions which organic compounds can undergo. The emphasis is upon the understanding of fundamental theories, and the author has shown his pedagogical ability in the manner in which the material is presented. Copies may be had at $3.60 from the Century Co., 353 Fourth Ave., N. Y.